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PHOTOSYNTHESIS OF PAPAYA AS AFFECTED BY LEAF MOSAIC
*John P. Decker and Marco A. Tió* 145

GROWTH CHARACTERISTICS OF GUINEA GRASS ON THE SEMIARID SOUTH COAST OF PUERTO RICO, AND THE EFFECT OF NITROGEN FERTILIZATION ON FORAGE YIELDS AND PROTEIN CONTENT
*José Vicente-Chandler and Jacinto Figarella* 151

LACK OF RESPONSE OF OLD COFFEE TREES GROWN IN ALONSO CLAY TO LIME AND PHOSPHATIC FERTILIZATION
*J. A. Bonnet, A. R. Riera, and M. A. Lugo-López* 161

YIELD RESPONSES TO DIFFERENT N-P-K LEVELS, AND CORRELATION WITH FOLIAR ANALYSIS, IN SAND-CULTURE STUDIES WITH CORN, SUGARCANE, AND COTTON
*J. A. Bonnet, A. R. Riera, and J. Roldán* 168

TERMITE-REPELLENT ACTIVITY AND CHEMICAL COMPOSITION OF WEST INDIAN MAHOGANY WOOD (*SWIETENIA MAHAGONI* JACQ.) WITH SPECIAL REFERENCE TO THE $P_2$ FRACTION
*Conrado F. Asenjo, Luis Amorós Marín, Wanda Torres, and Alice de Campillo* 185

FRUIT AND VITAMIN C PRODUCTION OF 5- AND 6-YEAR-OLD ACEROLA TREES.....*George C. Jackson and William Pennock* 196

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# THE JOURNAL OF AGRICULTURE
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Vol. XLII July 1958 No. 3


# Photosynthesis of Papaya as Affected by Leaf Mosaic


*John P. Decker*[1] *and Marco A. Tió*[2]


### INTRODUCTION

In southern and southwestern Puerto Rico, papaya (*Carica papaya*) is subject to a mosaic disease that reduces growth and yield so seriously as to limit commercial development of the crop. The disease is caused by a virus that is carried by the green citrus aphid (*Aphis spiraecola*, Patch) (*1*)[3].

Leaves of diseased plants (fig. 1) are irregularly mottled with light yellowish-green, are small in size, and often deformed to the degree of being distinctly filiform. Although photosynthetic capacity of mottled leaves would be expected to be subnormal, experimental evidence of the impairment is lacking and is the subject of this report.

### METHODS AND MATERIALS

The apparatus (fig. 2) used for measuring apparent photosynthesis was essentially a duplicate of one described earlier (*2*). It consisted of a leaf chamber, a small air pump, and an infrared gas analyzer in closed series.

The leaf chamber was the top of a 15-cm. petri dish that rested on a horizontal base of rigid sheet iron. The dish had notches cut in the rim to accommodate a petiole and a thermometer. Air-sampling and return tubes were fixed in the chamber base. Air temperature in the chamber was regulated by manual control of the flow of cold tapwater through loops of copper tubing soldered on the underside of the base plate.

Illumination was provided by a 300-watt internal-reflector spot lamp fixed rigidly 20 cm. above the chamber. A flat-bottomed glass dish containing 50 mm. of cold water served as a heat filter between lamp and leaf chamber. Water in the filter was cooled by a loop of copper tubing through which cold tapwater flowed. A single sheet of thin white paper (onion skin)


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[3] Italic numbers in parentheses refer to Literature Cited, p. 150.

FIG. 1.—A, Papaya leaf displaying typical symptoms of papaya mosaic; B, normal papaya leaf.

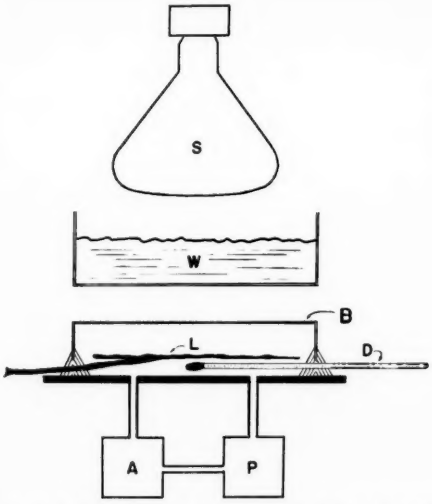


FIG. 2.—Diagram of the apparatus for measuring photosynthesis used in this work. Intact leaf (L) is sealed under glass dish (B) with modeling clay. Air is pumped (P) from chamber to analyzer (A) and back. Leaf is illuminated by overhead spot-lamp (S). Glass dish of water (W) acts as a heat filter. Thermometer (D).

was placed on top of the chamber as a diffuser. Light intensity ranged from 4,400 foot-candles at the center of the chamber to 2,400 near the edges.

The air pump was of the pulsating diaphragm type (Thiberg No. 1). It circulated air between chamber and analyzer at a rate of about 1,000 ml. per minute.

The gas analyzer was a model 15A Liston-Becker. It indicated continuously the $CO_2$ concentration of the system. The sample stream of air returned unaltered to the leaf chamber. The rate of decrease of concentra-

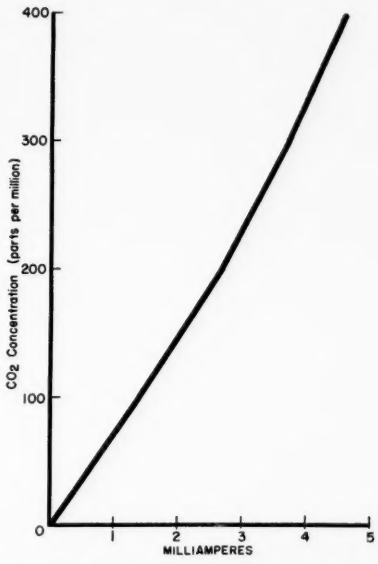


FIG. 3.—Calibration curve utilized for the infrared gas analyzer.

tion in the system was a direct measure of rate of uptake of $CO_2$ by a leaf, that is, of apparent photosynthesis.

The analyzer was calibrated as follows: Zero was set as the equilibrium reading obtained by recycling air through a system consisting of the analyzer, pump, and a scrubbing bottle of aqueous NaOH. The sensitivity control was then set so that a reference gas (compressed air containing about 344 p.p.m. of $CO_2$) gave a convenient reading, 4.1 ma. Next a recycling system consisting of a 2,400-ml. flask, pump, analyzer, and scrubber was set up and was run for about 20 minutes, 10 minutes after the analyzer indicated no $CO_2$ in the system. The scrubber was removed and the system

was run for several minutes to test for last traces of $CO_2$. The pump was stopped. A pre-set syringe fitted with a 27-gage needle was used to inject 0.251 ml. of $CO_2$ directly into the closed system through thick-wall rubber tubing. The pump was started and the equilibrium reading taken. The volume of the system was 2,510 ml.; thus, injections increased concentration in steps of 100 p.p.m.

The procedure was repeated and a calibration curve was drawn (fig. 3). Although the analyzer was slightly sensitive to water vapor (the difference between readings for dry and saturated nitrogen was equivalent to 20 p.p.m. of $CO_2$), measurements of changes of $CO_2$ concentration are free of error due to changes of humidity because the system remained constantly saturated, or nearly so.

The routine operating procedure was as follows: A leaf still attached to the plant was sealed in the chamber with modeling clay and left, with the system closed and operating, for 10–20 minutes at 32° C. and about 4,000 ft.-c. before measurements were started. An excess of $CO_2$ was added. Surges caused by the rapid introduction of $CO_2$ smoothed out in about 1 minute, then concentration began to fall smoothly and rapidly as the leaf removed $CO_2$ from the system. The fall was timed over a fixed interval of concentration, and the procedure was repeated until the apparent photosynthetic rate became constant.

Quintuplicate runs were made with 10 leaves of 5 healthy plants and with 10 leaves of 5 diseased plants. The plants were potted seedlings about 4 months old and from 50 to 60 cm. tall. They had grown in 15-cm. clay pots under natural light in a greenhouse. On December 6, 1956, some were inoculated by abrasion of leaves with carborundum powder and an inoculum extracted from diseased leaves. At the time of the experiments reported here (February 5 to 7, 1957) all inoculated plants showed characteristic symptoms of papaya mosaic, and all control plants appeared normal.

Leaf area was computed from the weight of a piece of heavy aluminum foil that had been cut to match the leaf. The leaf was flattened on the foil blank and stapled to hold it firmly while the foil was cut. The foil weighed 0.956 gm./dm$^2$.

Results were computed in terms of micrograms of $CO_2$ consumed per second per square decimeter of leaf area. Each run represented consumption by the leaf of 55.31 $\mu$g. of $CO_2$ at a mean $CO_2$ concentration of 300 p.p.m. The constant was computed as follows: Density of $CO_2$ at the calibration temperature of 27° C. is 1.7989 mg./l., and 1 p.p.m. is equivalent of 1.8 $\mu$g./l. From the calibration curve (fig. 3), the concentration interval 200–400 p.p.m. is equivalent to a meter interval of 1.95 ma. The meter interval for each run was 0.7 ma., thus a run represented a decrease of 55.31 $\mu$g. in the 428-ml. system ($0.7 \times {}^{200}/_{195} \times 1.8 \times 0.428 = 55.31$).

## RESULTS AND DISCUSSION

Photosynthetic rates (in μg./sec./dm²) ranged from 0.32 to 2.03 for diseased plants and from 2.30 to 3.46 for normal plants (table 1). The mean

TABLE 1.—*Apparent photosynthesis of mosaic and normal leaves of papaya*[1]

| Plant and leaf no. and condition | Leaf area | Time | Apparent photosynthesis |
|---|---|---|---|
| | *Dm.²* | *Sec.* | *μg. $CO_2$/sec./dm.²* |
| Mosaic | | | |
| 1-1 | 0.431 | 301 | 0.43 |
| 1-2 | .811 | 211 | .32 |
| 2-1 | .497 | 110 | 1.01 |
| 2-2 | .321 | 180 | .96 |
| 3-1 | .551 | 122 | .82 |
| 3-2 | .321 | 139 | 1.24 |
| 4-1 | .544 | 50 | 2.03 |
| 4-2 | .372 | 199 | .75 |
| 5-1 | .399 | 79 | 1.75 |
| 5-2 | .350 | 129 | 1.23 |
| Normal | | | |
| 1-1 | .686 | 35 | 2.30 |
| 1-2 | .657 | 36 | 2.34 |
| 2-1 | .482 | 39 | 2.94 |
| 2-2 | .363 | 44 | 3.46 |
| 3-1 | .552 | 35 | 2.86 |
| 3-2 | .576 | 35 | 2.77 |
| 4-1 | .645 | 28 | 3.06 |
| 4-2 | .650 | 26 | 3.27 |
| 5-2 | .356 | 52 | 2.99 |
| 5-2 | .608 | 27 | 3.37 |

[1] Times given are means of quintuplicate observations.

rate for diseased plants was 36 percent of that for normal plants. Results varied widely because diseased leaves ranged from nearly normal to acutely abnormal.

Impairment of growth and development associated with papaya mosaic is evidently caused, at least in part, by a reduction of photosynthetic capacity.

## SUMMARY

Comparative studies were made of the photosynthetic rates of leaves on normal papaya plants and of leaves on plants showing characteristic symptoms of papaya mosaic, a serious disease in southern Puerto Rico. The mean rate for diseased leaves was 36 percent of that for normal leaves.

## RESUMEN

Se hicieron estudios comparativos sobre las proporciones de fijación fotosintética en las hojas normales del papayo y en las hojas que demonstraban tener sintomas características del mosaico del papayo, la cual es una enfermedad grave en el sur de Puerto Rico. La proporción media de fijación para las hojas enfermas fué un 36 por ciento comparada con la de las hojas normales.


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# Growth Characteristics Of Guinea Grass on the Semiarid South Coast Of Puerto Rico, and the Effect Of Nitrogen Fertilization on Forage Yields and Protein Content

*José Vicente-Chandler and Jacinto Figarella*[1]

## INTRODUCTION

There is little effective moisture available during the 5-month dry season (December to April) which affects the south coast of Puerto Rico, and the nonirrigable lands of this area are best suited for use as permanent pastures. Much of this land is in Guinea grass (*Panicum maximum*), and the evidence available at present indicates that it is the best forage for this region.

The steepness of most of this land limits the use of silage; the unpredictable rainfall and other factors make the use of hay impractical. Cattle must therefore subsist during the critical dry season largely on the forage carried over in the field from the wet period. Since pasturage is abundant during the rainy season, the quantity and quality of this "carry-over" forage generally limits the stocking level and therefore the over-all productivity of livestock farms in this region.

The soils of the south coast are generally fertile and nitrogen seems to be the main limiting nutrient for forage crops. There is very little information available, however, on the effect of nitrogen fertilization upon Guinea grass in this region.

## OBJECTIVE

This study was carried out to determine the effect of nitrogen fertilizer applied to a closely grazed Guinea grass pasture toward the end of the wet season on the yield and protein content of the forage produced for "carry-over" into the dry season. The changes in the protein content of the forage as it dried out in the field and the effect of the treatments on the forage produced during the subsequent rainy season were also studied.

## MATERIALS AND METHODS

The rainfall data available for the south coast of Puerto Rico were examined to determine the rainfall pattern of this area and provide a basis

[1] Project Supervisor and Soil Scientist, respectively, of a research project cooperative between the Agricultural Research Service of the United States Department of Agriculture and the Agricultural Experiment Station of the University of Puerto Rico, Río Piedras, P. R.

for predicting when the dry season was likely to start. A record of rainfall was kept at the experimental site near Coamo, P. R., where conditions typical of the semiarid south coast prevail.

The area selected for the experiment had an excellent stand of Guinea grass and since the soil was well provided with phosphorus and potassium, no fertilizer was applied other than nitrogen as called for by the treatments. The soil is a deep, sloping Coamo clay. The surface soil has a pH of 6.2, a total exchange capacity of 35.4 m.e. per 100 gm. of soil, a total exchangeable base content of 29.4 m.e. per 100 gm. of soil, and an organic-matter content of 3.4 percent, with a C:N ratio of about 10.

A preliminary study was carried out during the first rainy season, when moisture was abundant, to determine the effect of stage of growth on protein content and on the dry-matter and protein yields of Guinea grass when heavily fertilized with nitrogen. The grass in a 40-x-40-foot area was cut and 400 pounds per acre of nitrogen were applied in the form of ammonium sulfate. Four plots 4 x 4 feet in area were harvested every 20 days. The forage was weighed and dried and its protein content determined.

Another preliminary study was carried out starting on November 1, 1953, to determine the changes in the protein content of various portions of the Guinea grass plant as it grew and then dried out in the field. The forage was cut back and 400 pounds of nitrogen in the form of ammonium sulfate were applied per acre. Six plants were dug up at monthly intervals and were divided into roots, stems, leaf sheaths, and leaves. These were analyzed to determine their protein content.

On November 1, 1953, the forage in the experimental area was cut back and the treatments—0, 100, 200, and 400 pounds of nitrogen per acre in the form of ammonium sulfate—applied. All treatments were replicated four times. Plots were 10 x 20 feet in size with a 2-foot buffer zone around each.

Forage samples were taken from all plots at monthly intervals and analyzed to determine their nitrogen content. In addition, composited samples for all treatments were analyzed for soluble nitrogen. All plots were harvested toward the end of the dry season, May 1, and twice during the following rainy season, and yields and protein determinations were made. The experiment was repeated in the same manner starting on November 1, 1954.

## RESULTS

### THE RAINFALL PATTERN OF THE SOUTH COAST

The average monthly rainfall at seven locations throughout the south coast is shown in table 1. There does not appear to be much difference in the over-all pattern of rainfall in this area, with the exception of Lajas

where precipitation seems to be better distributed throughout the year than at other locations. Average annual rainfall for this region is about 36.5 inches with almost all the effective precipitation (about 29 inches) falling during the 7-month period of May to November. The inch or so of monthly rainfall which can be expected during the remainder of the year produces very little plant growth since it occurs largely in the form of dew or very light showers at a time when climatic conditions favor very rapid evaporation.

TABLE 1.—*10-year average rainfall by months at 7 locations throughout the semiarid south coast of Puerto Rico*

| Month | Aguirre | Potala | Coamo | Santa Isabel | Yauco | Ensenada | Lajas | Average |
|---|---|---|---|---|---|---|---|---|
| January | 1.40 | 0.78 | 1.18 | 1.12 | 0.92 | 0.79 | 1.40 | 1.08 |
| February | 1.48 | 1.18 | 1.11 | 1.06 | 1.54 | 1.42 | 1.64 | 1.35 |
| March | 1.19 | .92 | .63 | .87 | 1.14 | 1.28 | 3.18 | 1.32 |
| April | 1.96 | 2.00 | 1.78 | 1.55 | 1.84 | 1.82 | 2.13 | 1.87 |
| May | 4.40 | 4.16 | 4.54 | 3.82 | 5.54 | 3.58 | 2.02 | 4.01 |
| June | 4.48 | 2.72 | 2.89 | 2.96 | 2.65 | 2.25 | 2.11 | 2.87 |
| July | 4.12 | 2.65 | 2.17 | 2.54 | 2.38 | 2.00 | 5.16 | 3.00 |
| August | 4.90 | 4.09 | 5.12 | 3.85 | 4.86 | 3.06 | 4.63 | 4.36 |
| September | 6.06 | 5.44 | 6.18 | 4.89 | 6.02 | 4.62 | 5.95 | 5.59 |
| October | 5.93 | 5.18 | 5.70 | 5.61 | 4.92 | 4.26 | 3.94 | 5.08 |
| November | 4.51 | 3.77 | 4.07 | 3.94 | 3.83 | 3.84 | 4.30 | 4.04 |
| December | 1.84 | 1.10 | 1.68 | 1.53 | 1.34 | 1.52 | 4.54 | 1.92 |
| Total | 42.27 | 33.99 | 37.05 | 33.74 | 36.98 | 30.44 | 41.00 | 36.49 |

## THE GROWTH PATTERN OF GUINEA GRASS

The effect of stage of growth on the dry-matter and protein yields and protein content of heavily fertilized Guinea grass growing with abundant moisture is shown in figure 1.

These data show that Guinea grass grows rapidly for a period of about 80 days after which its growth rate declines. The protein content of the forage decreases rapidly with age. Yields of crude protein increase with age up to about 80 days after which they remain about constant, the rise in yield after this date, apparently compensating for the continued drop in protein content.

From the above it appears that, in order to obtain a large quantity of carry-over forage of a desirable protein content for use during the dry season, the growth of Guinea grass should be stopped by drouth when it is between 60 and 80 days of age. Thus, for this purpose, pastures should be cut or grazed closely and fertilizer applied in October or early November.

Before growth is stopped by drouth the grass can make use of 6 to 11 inches of rainfall which can be expected during the remainder of the calendar year and which is sufficient to allow the grass to grow for a desirable period.

## EFFECT OF MATURITY ON THE PROTEIN CONTENT OF SECTIONS OF THE GUINEA GRASS PLANT

As will be shown later, traces only of soluble nitrogen were found in the forage. The assumption is therefore made that the nitrogen found by analysis is present in amino form and nitrogen multiplied by 6.25 is reported as protein.

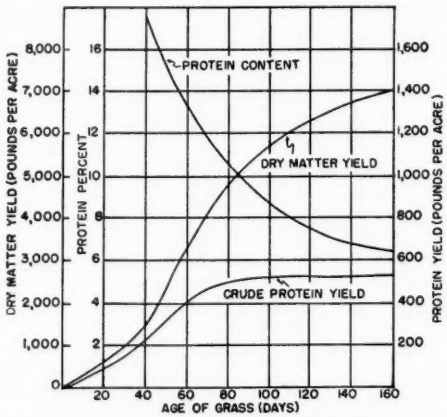


FIG. 1.—Variation with age in the dry-matter and protein yields and protein content of heavily fertilized Guinea grass growing with abundant moisture.

The data in table 2 show the changes occurring with age in the protein content of sections of heavily fertilized Guinea grass when carried over into the dry season. The protein content of the total forage decreased rapidly with age. That of the roots, stems, and leaf sheaths, however, did not vary much over the 6-month period of the study. On the other hand, the protein content of the leaves, although always higher than that of the other sections, dropped steadily with age from 24.4 percent, when the grass was 1 month old, to 14.6 percent at an age of 6 months. It seems that the drop with age in the protein content of the total forage was partly caused by the decrease in the protein content of the leaves, but more so by an increase in the proportion of stems and leaf sheaths.

## THE EFFECT OF NITROGEN FERTILIZATION

The results of the experiment carried out to determine the effect of nitrogen fertilization on the yield and protein content of Guinea grass are

summarized in table 3. During the first year weather conditions were about normal and the plots received sufficient rainfall after being cut back and fertilized so that the grass produced an abundance of forage for carrying over into the dry season. During the second year the drouth started shortly after the trials were initiated and seriously limited the quantity of carry-over forage. The data shown are therefore representative of what can be expected under both these conditions. The first year's results are discussed in more detail since they represent normal conditions.

*Carry-over forage*

The quantity of dry matter produced during the first year for carry-over into the dry season was increased significantly, from 3,555 to 5,520 pounds

TABLE 2.—*Changes with maturity in the percentage protein content of different sections of Guinea grass cut and fertilized with 400 pounds of nitrogen per acre on November 1, 1953*[1]

| Date | Roots | Stem | Leaf sheath | Leaves | Total forage (above-ground portions) |
|---|---|---|---|---|---|
| Dec. 1 | 7.2 | 9.2 | 10.1 | 24.4 | 22.7 |
| Jan. 1 | 6.9 | 10.6 | 9.1 | 18.5 | 13.4 |
| Feb. 1 | 7.8 | 9.6 | 8.7 | 15.8 | 10.5 |
| Mar. 1 | 7.3 | 11.0 | 8.8 | 15.6 | 10.7 |
| Apr. 1 | 6.8 | 7.4 | 8.8 | 13.8 | 9.4 |
| May 1 | 7.1 | 8.4 | 8.9 | 14.6 | 9.3 |

[1] All values are averages of 6 samples.

per acre, by an application of 100 pounds of nitrogen per acre. Heavier applications of nitrogen had no apparent effect on yields. During the second year the drouth which started soon after the treatments were applied greatly curtailed plant growth, and nitrogen fertilization had no significant effect on yields.

Nitrogen fertilization over both years had a marked effect on the protein content of the forage carried over into the dry season and allowed to dry in the field. The protein content of the forage tended to increase with each increment of nitrogen fertilization up to the highest level tested (400 pounds per acre). This effect was apparent at all ages, although the protein content in all cases dropped rapidly as the grass matured and dried out. Application of 100 pounds of nitrogen per acre increased the protein content of the herbage significantly at all ages during both years.

At 1 month the protein content of heavily fertilized Guinea grass was comparable to that of good legume hay. At the end of the dry season of the first year, when the grass was about 6 months old, the untreated forage had

TABLE 3.—*The effect of nitrogen fertilization on the yield (lb./A.) and protein content (percent) of a Guinea grass pasture harvested at different times over a 1-year period following application of the fertilizer, 1953–55*[1]

| Season, and nitrogen fertilization (lb./A.)[2] | Protein content on— | | | | Harvested May 1 | | | Harvested September 1 | | | Harvested November 1 | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dec. 1 | Jan. 1 | Feb. 1 | Mar. 1 | Protein content | Dry-matter yields | Protein yields | Protein content | Dry-matter yields | Protein yields | Protein content | Dry-matter yields | Protein yields | Protein content | Dry-matter yields | Protein yields |
| 1953–54 | | | | | | | | | | | | | | | | |
| 0 | 14.0 | 10.6 | 7.5 | 7.4 | 6.7 | 3,555 | 238 | 7.3 | 2,340 | 170 | 5.2 | 3,815 | 200 | 6.3 | 9,710 | 608 |
| 100 | 19.4 | 14.2 | 10.8 | 10.0 | 8.8 | 5,520 | 486 | 7.2 | 2,555 | 185 | 5.5 | 4,570 | 250 | 7.3 | 12,645 | 921 |
| 200 | 20.3 | 14.9 | 12.0 | 11.8 | 9.2 | 4,690 | 432 | 8.6 | 2,660 | 230 | 5.3 | 5,005 | 265 | 7.5 | 12,335 | 927 |
| 400 | 21.7 | 15.2 | 13.3 | 13.3 | 10.9 | 5,155 | 562 | 11.5 | 2,530 | 290 | 6.1 | 5,005 | 305 | 9.1 | 12,690 | 1,157 |
| Inches of rainfall[3] | 4.04 | 1.92 | 0.26 | 2.95 | | 11.74 | | | 10.59 | | | 24.63 | | | 46.96 | |
| 1954–55 | | | | | | | | | | | | | | | | |
| 0 | 12.0 | 8.4 | 6.1 | 5.5 | 4.8 | 1,365 | 66 | 6.0 | 3,930 | 235 | 5.0 | 4,210 | 210 | 5.4 | 9,505 | 511 |
| 100 | 17.6 | 10.3 | 7.5 | 7.1 | 7.2 | 2,005 | 145 | 11.2 | 3,515 | 395 | 6.0 | 4,970 | 300 | 7.0 | 10,490 | 740 |
| 200 | 17.6 | 11.1 | 8.6 | 8.4 | 7.7 | 2,015 | 155 | 10.8 | 3,990 | 430 | 5.7 | 5,695 | 325 | 7.8 | 11,700 | 910 |
| 400 | 19.8 | 13.3 | 9.4 | 9.4 | 9.0 | 1,335 | 120 | 13.3 | 3,715 | 495 | 8.1 | 5,440 | 440 | 10.0 | 10,490 | 1,055 |
| Inches of rainfall[3] | 2.10 | 0.90 | 0.05 | 0.10 | | 3.74 | | | 11.10 | | | 16.55 | | | 31.39 | |

[1] All values are averages of 4 replications. The experiment was carried out at Coamo, under conditions typical of the semiarid south coast.

[2] Forage cut back and fertilizer applied Nov. 1, in both years.

[3] For the first 4 columns this is rainfall occurring during the previous month. In all other cases it is total rainfall over the yield period, entered under dry-matter yields.

a protein content of only 6.7 percent. That fertilized with 100 pounds of nitrogen had 8.8 percent protein while that fertilized with 400 pounds of nitrogen had 10.9 percent protein.

During the first year the application of 100 pounds of nitrogen per acre about doubled the quantity of protein carried over in the field, from 238 to 486 pounds per acre. Heavier applications of nitrogen did not significantly increase protein yields. Similar results were obtained during the second year.

*Residual effects*

The yields of dry matter during subsequent rainy seasons were not significantly affected by nitrogen fertilization. Total yields of dry matter produced during the first year, however, were significantly increased, from 9,710 pounds to 12,645 pounds per acre, by the application of 100 pounds of nitrogen per acre.

In general, nitrogen fertilization tended to increase protein yields and protein content of the forage produced during the residual yield periods. In some instances the effect of nitrogen fertilization was evident up to one year after application of the fertilizer. This residual effect of nitrogen fertilization was more marked during the second year when plant growth during the yield period immediately following application of the fertilizer was greatly curtailed by drouth.

During the first year the 400-pound level of nitrogen fertilizer significantly increased protein yields and protein content of the forage over the first residual yield period. This treatment as well as the 100-pound level of nitrogen significantly increased protein yields during the second residual-yield period.

The total protein yields during this year were significantly increased by the application of 100 pounds of nitrogen fertilizer, from 608 to 921 pounds per acre. The 400-pound level of nitrogen caused a further significant increase in protein yields to 1,157 pounds per acre, and also significantly increased the protein content of the forage produced during this year.

During the second year the 100-pound level of nitrogen fertilizer significantly increased protein yields and protein content of the forage produced during both residual-yield periods. A further significant increase was brought about in both instances by the 400-pound level of nitrogen.

The 100-pound level of nitrogen caused a significant increase in total protein yields, from 511 pounds per acre to 740 pounds, and in protein content, from 5.4 to 7.0 percent, of the forage produced during this second year. The 400-pound level of nitrogen caused a further significant increase in total protein yields to 1,055 pounds per acre, and protein content to 10 percent.

[3] For the first 4 columns this is rainfall occurring during the previous month. In all other cases it is total rainfall over the yield period, entered under dry-matter yields.

*Recovery of applied nitrogen*

Recovery in the forage of the applied nitrogen was rather low during both years. Highest recovery was at the 100-pound level of nitrogen with an average of about 50 percent during the first year and about 37 percent during the second. Over both years an average of only about 29 percent of the 200-pound application and only about 22 percent of the 400-pound application was recovered. It is possible that, had yields been taken over a longer period following application of the fertilizer, a higher proportion of the nitrogen would have appeared in the forage as protein.

*Soluble-nitrogen content of forage*

In no case did the forage samples analyzed contain more than 0.5 percent of soluble nitrogen and values were usually considerably below this level. Only traces of nitrites were found. There thus seems to be little danger of the forage ever having been dangerously high in nitrogen compounds which could be toxic to livestock, and almost all the protein was apparently in a form which could be utilized by ruminants for their nutrition.

## DISCUSSION OF RESULTS

The data obtained during the first year when rainfall conditions were normal show that, from a practical standpoint, the application of 100 pounds of nitrogen per acre was the most desirable. The amount of forage produced for carry-over into the critical dry season was increased 55 percent, from 3,555 to 5,520 pounds of dry matter per acre, by this treatment. Similarly, the protein content of the forage was increased 31 percent, from 6.7 to 8.8 percent, and protein yields 105 percent, from 238 to 486 pounds per acre. Residual yields of dry matter and protein tended to be increased by this treatment. Total dry-matter yields for the 1-year period were increased 30 percent, from 9,710 to 12,645 pounds per acre, and protein yields were increased 51 percent, from 608 to 921 pounds per acre. Furthermore, a much higher proportion of the nitrogen applied was recovered at this level of fertilization than with heavier applications of nitrogen.

Since forage is generally abundant during the wet season the stocking rate of farms is usually limited by the carrying capacity of the pastures during the dry season. Assuming 50-percent utilization of the forage by livestock and the consumption of 20 pounds of dry matter daily per head by mature animals, the carry-over forage produced by the unfertilized plots would provide only 88 animal-days of grazing during the 5-month dry season, as contrasted with 138 animal-days which would be provided by the plots receiving 100 pounds of nitrogen fertilizer. Furthermore, the higher protein content of the fertilized forage would in all probability improve animal performance. Thus, with proper fertilization and pasture

management, it is theoretically possible under the conditions of this experiment to maintain 1 head of livestock on every 1¼ acres throughout the critical dry season on carry-over forage. Such a level of stocking may be feasible where some silage or cane tops can be provided during the dry season but in general, 2 acres per head would seem to be a more practical goal. This compares favorably with the 3 to 4 acres required with Guinea grass pastures under present conditions.

The importance of careful management of these pastures cannot be overemphasized. Productivity of Guinea grass pastures in this area can be markedly increased by proper management alone. The pastures should not be overgrazed, especially toward the end of the wet season, and should be fenced to provide for rotational grazing, particularly during the dry season. Otherwise the animals will feed on the highly nutritious leaves during the beginning of the dry season and have only the less nutritious leaf sheaths and stems to graze on later. In order to obtain sufficient forage having a proper protein content for carry-over into the dry season, nitrogen fertilizer should be applied to closely grazed pastures during October or early November. These pastures should not be grazed again until forage becomes scarce during the dry season.

The results of this study indicate that livestock production in the nonirrigable, semiarid region of the Island can be appreciably increased by proper management and fertilization of Guinea grass pastures with nitrogen. Further increases may be obtained through the use of silage, which should be greatly expanded, but the potential for livestock production in this region appears to be generally limited by rainfall and topography.

## SUMMARY

The stocking level and hence the productive capacity of pastures in the nonirrigable, semiarid region of Puerto Rico is limited by the pasturage available during the approximately 5-month dry season of December to April. This, in turn, largely depends on the quantity and quality of the roughage carried over in the field from the wet season when an excess of forage is produced.

The application of 100 pounds of nitrogen per acre to a closely grazed Guinea grass pasture toward the end of the wet season, November 1, resulted in a marked increase in the dry matter from 3,555 to 5,520 pounds per acre of protein yields from 238 to 486 pounds per acre, and in the protein content from 6.7 to 8.8 percent, of the forage carried over in the field for grazing toward the end of the dry season.

Total yields of dry forage produced during the year following application of the fertilizer were increased from 9,710 to 12,645 pounds per acre. Total yields of protein were also increased by this treatment from 608 to 921

pounds per acre. About 50 percent of the nitrogen applied was recovered in the forage. Heavier applications of nitrogen tended to increase protein yields and also the protein content of the forage, but did not affect yields of dry matter.

Through good management and proper fertilization with nitrogen it appears possible markedly to increase the carrying capacity of Guinea grass pastures in this area.

**RESUMEN**

La producción de los pastos de yerba Guinea en la costa semi-árida de Puerto Rico depende en gran parte de la calidad y cantidad de forraje que pueda conservarse en el campo durante la época de lluvia, cuando abunda el pasto, para ser pastoreado luego durante la sequía.

Mediante la aplicación de 100 libras de nitrógeno por cuerda antes de comenzar la sequía se aumentó la cantidad de forraje seco conservado en el campo de 3,555 a 5,520 libras por cuerda, la cantidad de proteína de 238 a 486 libras por cuerda y el contenido de proteína del forraje de 6.7 a 8.8 por ciento. El rendimiento de forraje seco producido durante el año subsiguiente a la aplicación de nitrógeno también aumentó de 9,710 libras a 12,645 libras por cuerda. Asimismo la producción de proteína aumentó de 608 a 921 libras por cuerda. Se recobró en el forraje más o menos la mitad del nitrógeno aplicado. Aplicaciones mayores de nitrógeno aumentaron el contenido de proteína pero no el rendimiento de la yerba.

Mediante el manejo y abonamiento adecuado es posible aumentar considerablemente la producción de los pastos de yerba de Guinea en la región semi-árida de Puerto Rico.

# Lack of Response of Old Coffee Trees Grown in Alonso Clay To Lime and Phosphatic Fertilization[1]

*J. A. Bonnet, A. R. Riera, and M. A. Lugo-López*[2]

## INTRODUCTION

Lime applications to the acid tropical soils of Puerto Rico have been shown to be beneficial to sugarcane (*1*)[3], forage crops (*2*), fiber crops (*11*), and such food crops as sweetpotatoes and corn (*4, 11*), but few results have been obtained with coffee. McClelland reported that no appreciable effects on the growth (*12*) or production (*13*) of coffee trees could be measured after liming an acid Catalina clay. Later studies by Guiscafré (*5*) and Guiscafré and Gómez (*6, 7, 8*), made between 1936 and 1940, of two acid soils, indicated that limestone applications at rates of ¾ ton to 3 tons to the acre did not affect coffee yields in soils with pH values from 5.5 to 6.0. In soils with pH values from 4.0 to 4.5, limestone applied at the rate of 1 ton to the acre produced significant yield increases, but 2, 3, or 4 tons did not.

Information regarding the fertilizer requirements of coffee is just as meager. McClelland reported (*13*) that potash application was essential for soils wherein coffee grows, but phosphorus was not. A report issued in 1946, by Gómez *et al.* (*9*) covering the results of a 6-year fertilizer test with 16-year-old coffee trees grown on a Catalina soil, emphasized the need of nitrogen and phosphorus, but not of potassium. Gómez' group also reported (*9*) that phosphorus applications were necessary for maximum yields in a 3-year test with young coffee plants on the same soil type at Mayagüez.

This paper reports data obtained from a 4-year experiment with old coffee trees that received lime and phosphatic fertilizers. The inherent limitations of this work are recognized and will be discussed.

[1] Joint contribution of the Department of Soils, the Central Analytical Laboratory, and the Castañer and Gurabo Agricultural Experiment Substations.

[2] Head of the Department of Soils, Associate Chemist in Charge of the Central Analytical Laboratory, and Associate Soil Scientist in Charge of the Gurabo Substation, Agricultural Experiment Station, University of Puerto Rico, Río Piedras, P. R. Appreciation is expressed to S. Rodríguez, Assistant Agronomist in Charge, and to J. Toro and L. Alemañy, Research Assistants in Agronomy, Castañer Substation, for their help in harvesting and supervising the fieldwork. J. Roldán, Assistant Chemist of the Soils Department, helped in the initial stages of the work. G. Malaret Juarbe, former Research Assistant in Chemistry, performed some laboratory analyses. Thanks are expressed to Compagnie Nord Africaine de L'Hyperphosphate Reno, 47 Rue de Liege, Paris, and to their agent, Bernard Carreau, at Fort de France, Martinique, for supplying the superphosphate.

[3] Italic numbers in parentheses refer to Literature Cited, pp. 166–7.

## MATERIALS AND METHODS

The experiment was located at the Agricultural Experiment Substation Farm in the vicinity of Adjuntas and Lares, which lies in the humid, mountainous heart of the Coffee Region of Puerto Rico. The soil is Alonso clay, a rather extensive acid (pH 4.2) upland latosol derived from reddish-purple igneous rocks (*15*). In this soil feldspars of the oligoclase variety are in medium abundance in the fine sand and silt fractions, and kaolinite is the predominant clay mineral (*10*). The exchange capacity ranged from 20 to 40 m.e. per 100 gm. of soil, unusually high for latosols. The slope of the field ranged anywhere from 20 to 40 percent.

The experiment was laid out following a paired-plot design including 16 treatments: Two sources of phosphates—super- and hyperphosphate[4]—each was used at four rates, either with or without lime: 0, 50, 125, and 200 pounds of $P_2O_5$ to the acre. A single tree represented a plot with an area of 8 feet by 9 feet; 1 acre contained 605 trees, probably between 10 and 15 years of age. Each treatment was replicated 15 times for a total of 240 trees in the experiment. Lime was applied to those plots requiring it according to the experimental design, at a rate of 4 tons to the acre (soil pH 4.2), April 14–17, 1953. The plots were fertilized May 21, 1953, with 108 pounds of nitrogen and 60 pounds of $K_2O$, on the acre basis, and phosphoric acid was determined by treatment differentials. The lime and fertilizer were evenly distributed in the canopy area. The three succeeding crops were fertilized as was the 1953–54 crop, but the lime applications were not repeated. The berries were harvested for four consecutive crop years and production figures are reported as marketable coffee by applying a standard conversion factor to the yield of coffee berries obtained.

A soil profile was sampled to a 6-foot depth in the experimental area and the phosphate levels determined according to the Olsen bicarbonate method (*14*).

## RESULTS AND DISCUSSION

Table 1 presents data on yields of marketable coffee for four succeeding crops. Table 2 provides a direct and simple comparison of the coffee production by limed and unlimed plots. No significant differences were measured which could be attributed to either type or rate of phosphatic fertilizer, irrespective of the presence of absence of lime. Alonso soil is apparently

[4] A phosphatic fertilizer obtained from the North African sedimentary deposits of phosphate of lime. Chemical analyses revealed that hyperphosphate had an average content of 26-percent total $P_2O_5$ while the commonly used superphosphate has only 19 percent. The available $P_2O_5$, however, was only 5.9 percent in the hyperphosphate while almost all is available in the superphosphate.

TABLE 1.—*Effect of phosphatic fertilizers applied to acid Alonso clay, in the absence or presence of lime, upon marketable coffee yields*

| Treatment | Mean[1] hundredweights per acre of coffee at indicated $P_2O_5$ levels, in pounds per acre | | | |
|---|---|---|---|---|
| | 0 | 50 | 125 | 200 |
| *1953–54 crop* | | | | |
| Superphosphate | 7.22 | 5.98 | 6.50 | 7.60 |
| Hyperphosphate | 6.27 | 6.03 | 4.99 | 7.74 |
| Superphosphate plus lime | 7.56 | 6.59 | 6.81 | 6.59 |
| Hyperphosphate plus lime | 7.18 | 7.79 | 5.43 | 6.19 |
| *1954–55 crop* | | | | |
| Superphosphate | 2.26 | 1.63 | 1.70 | 1.50 |
| Hyperphosphate | 1.38 | 2.67 | 1.27 | 1.67 |
| Superphosphate plus lime | .78 | 1.49 | 1.80 | 1.18 |
| Hyperphosphate plus lime | 1.58 | 1.43 | 1.02 | 2.89 |
| *1955–56 crop* | | | | |
| Superphosphate | 7.59 | 7.87 | 9.57 | 8.34 |
| Hyperphosphate | 6.87 | 6.99 | 5.50 | 7.17 |
| Superphosphate plus lime | 7.30 | 7.09 | 4.91 | 7.52 |
| Hyperphosphate plus lime | 4.56 | 6.96 | 6.86 | 3.59 |
| *1956–57 crop* | | | | |
| Superphosphate | 3.00 | 3.49 | 2.30 | 1.81 |
| Hyperphosphate | 3.34 | 3.75 | 2.18 | 1.92 |
| Superphosphate plus lime | 1.13 | 1.12 | 2.81 | 2.36 |
| Hyperphosphate plus lime | .96 | 2.36 | 2.44 | 2.47 |

[1] No significant differences between treatment means.

TABLE 2.—*Summary of the effect of liming acid Alonso clay upon yields of marketable coffee, 1953–57*

| Crop | Mean yields under indicated treatment | | L.S.D. at level indicated | |
|---|---|---|---|---|
| | Unlimed | Limed | 5 percent | 1 percent |
| | *Cwt./A.* | *Cwt./A.* | | |
| 1953–54 | 6.83 | 6.89 | 1.25 | 1.69 |
| 1954–55 | 1.77 | 1.32 | .72 | .95 |
| 1955–56 | 8.34 | 6.70 | 2.07 | 2.74 |
| 1956–57 | 2.65 | 1.85 | 1.32 | 1.75 |

well supplied with available phosphorus, as shown in the following tabulation:

| *Depth in inches* | $P_2O_5$, *lb./A.* |
|---|---|
| 0–6 | 18.3 |
| 6–12 | 16.5 |
| 12–18 | 11.0 |
| 18–30 | 7.4 |
| 30–42 | 36.6 |
| Total | 89.8 |

Coffee trees can obtain almost 35 pounds of available phosphorus from the upper acre-foot of this soil. Moreover, the 12- to 42-inch section of the profile can contribute an additional 55 pounds through the activity of feeding roots that penetrate to exploit a larger volume of soil. The profile as a whole can contribute a total of approximately 90 pounds to the acre, which seems to be more than ample for old coffee trees under the conditions prevalent in the experiment herein reported.

Thus, applications of phosphatic fertilizers to this soil are in all probability unnecessary, at least for the production of four crops of coffee, as shown under the experimental conditions herein reported. These data are in agreement with those of McClelland (*13*), who did not obtain responses from phosphatic fertilization in the Mayagüez area. Gómez *et al.* (*9*) measured responses from phosphorus on Catalina clay in the Lares area. The difference in the phosphorus-supplying power of the soils, as well as the previous management of the groves, might account for such apparent discrepancies.

Studies (*3*) with superphosphate tagged with $P^{32}$ applied to a nearby field of Alonso clay in the same Substation farm, indicated that the coffee trees absorbed most of the required phosphorus from the soil.

The application of lime at a rate of 4 tons to the acre produced no effects, either detrimental or beneficial, upon the yields of marketable coffee. These results are completely in agreement with those obtained by McClelland (*13*) in his liming experiments from 1913 to 1917 in the Mayagüez area. His results are summarized in the following tabulation, expressed as yields per tree, in liters:

| *Year* | *Unlimed* | *Limed* |
|---|---|---|
| 1914 | 1.23 | 1.33 |
| 1915 | 1.85 | 1.55 |
| 1916 | 1.33 | 1.69 |
| 1917 | 2.38 | 2.29 |

Other investigators (*5*, *6*, *7*, *8*) obtained somewhat similar results to those mentioned previously when lime was applied to slightly acid soils.

The same workers obtained increases in coffee yields following applications of 1 ton of lime to the acre to more acid soils, but failed to observe any effects when more lime was used.

The trees used in this work were old. Responses of young coffee trees to phosphoric acid were observed in a 3-year experiment at Mayagüez (*9*). However, the fact that Alonso clay appears to be well supplied with available phosphates might perhaps preclude any possibility of response to phosphatic fertilizers, even in young trees.

The use of lime in coffee groves is generally unnecessary unless high soil acidity develops, as when the pH values are in the vicinity of 4 or 4.5. Even then lime seems beneficial only in small doses of 1 ton to the acre, the larger doses exerting no influence upon crop yields (*8*).

The shortcomings of this work—mainly arising from the use of old coffee trees and because of the fact that the Alonso clay is rather unusual among the soils whereon coffee is grown commercially because of its high fertility level—must be recognized. In spite of such limitations, the results obtained provide a key to what to expect from lime and phosphatic fertilization of old coffee groves established in Alonso clay soils.

## SUMMARY

Data are reported here from a 4-year field experiment in an Alonso clay with old coffee trees in the vicinity of Adjuntas and Lares, in the heart of the Coffee Region of Puerto Rico. The experiment included super- and hyperphosphate applied either with or without lime, and at rates of 0, 50, 125, and 200 pounds to the acre. Lime was applied at a rate of 4 tons to the acre. No significant differences were measured in coffee yields which could be attributed to any of the treatments. These results are analyzed in the light of previous similar work. The general statement could be made that lime applications to acid Alonso clay soils do not affect the yields of coffee in any way, except perhaps when the acidity is high (pH 4.0 to 4.5), in which case applications of 1 ton are apparently sufficient. There is no need to apply phosphatic ferilizers to Alonso soils. However, the beneficial effects of these fertilizers have been demonstrated when dealing with other soils.

## RESUMEN

Este trabajo presenta datos obtenidos de un experimento de campo que duró cuatro años y llevado a cabo en un suelo del tipo Alonso arcilloso con árboles viejos de café en la vecindad entre Adjuntas y Lares. Puede decirse que este sitio queda en el mismo centro de la zona cafetalera de Puerto Rico.

Los tratamientos a los cuales se sometió este campo experimental in-

cluyeron el uso del superfosfato y el hiperfosfato, con y sin cal, en las proporciones siguientes: 0, 50, 125 y 200 libras por acre. La cal se aplicó a razón de 4 toneladas por acre.

No se obtuvieron diferencias significativas en los rendimientos de los árboles de café que pudieran atribuírse a los efectos de los distintos tratamientos. Estos resultados fueron analizados a la luz de los anteriormente obtenidos por otros investigadores de otros trabajos similares al que aquí se informa.

Se puede adelantar, en términos generales, que la aplicación de cal a suelos ácidos, como el Alonso arcilloso, no afecta significativamente los rendimientos de los árboles de café. Puede ser que en otros suelos mucho más ácidos (pH menor de 4.0 a 4.5) la aplicación de una tonelada de cal por acre, que sería más que suficiente, afectara favorablemente los rendimientos.

Se demostró que a suelos, como este Alonso arcilloso, no hay necesidad de aplicarles abonos fosfatados para aumentar los rendimientos del café, sin embargo, hay evidencia obtenida anteriormente que estos abonos han resultado beneficiosos cuando se han aplicado a otros suelos.

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# Yield Responses to Different N-P-K Levels, and Correlations With Foliar Analysis, in Sand-Culture Studies With Corn, Sugarcane, and Cotton[1]

*J. A. Bonnet, A. R. Riera, and J. Roldán*[2]

## INTRODUCTION

A research project to study the nutrient requirements of the principal economic crops of Puerto Rico was started in January 1940. The sand-culture method, with ample space and suitable conditions for normal root development of field crops, was used. Inert sand rather than soil was preferred because the sand offered the means to control the initial quantity of nutrients added per treatment to study the minimum to maximum N-P-K levels that may cause deficiency to excess for crop yields.

## EQUIPMENT AND METHODS

Twenty-eight concrete pits were constructed (fig. 1) each about 0.001 acre in area, 10 feet 5/8 inch long, 4 feet 4 inches wide, and from 1 foot 9 inches to 1 foot 11 inches in depth, with drainage outlets. The pits, ditches, and walks occupied the floor space over which a glasshouse (fig. 2), 75 feet 4 1/2 inches long by 35 feet 2 inches wide, and 10 feet in height at the eaves to 19 feet at top center, was placed. The house was surrounded by a 3-foot concrete wall except for the double doors at the front and back. The rest of the house was a steel framework covered with glass plates containing movable ventilators at both sides and at the top. The openings were screened with 16-mesh copper wire gauze to protect the crop from insect pests.

Rainwater collected from the roof of the greenhouse and stored in two

[1] The results presented in this paper cover the work of a research project included in the 15-year (1940–55) program assigned to the Soils Department. The first 8 years devoted to this project were of an exploratory nature until normal crop growth was obtained. Failures and hopes alternated, then Eureka! The authors wish to express their appreciation to Director Arturo Roque of this Station, who, since he took charge of administrative duties in 1943, strengthened our spiritual faith in the accomplishments of the objectives of this project with stimuli and budget-support. The authors wish also to express their appreciation to Associate Director for Research B. G. Capó, for his guidance and technical advice on the organization, planning, and execution of this project. We wish likewise to thank Ishver S. Bangdiwala, Associate Statistician, Head of the Statistics Department, for his collaboration on the statistical interpretation of the data.

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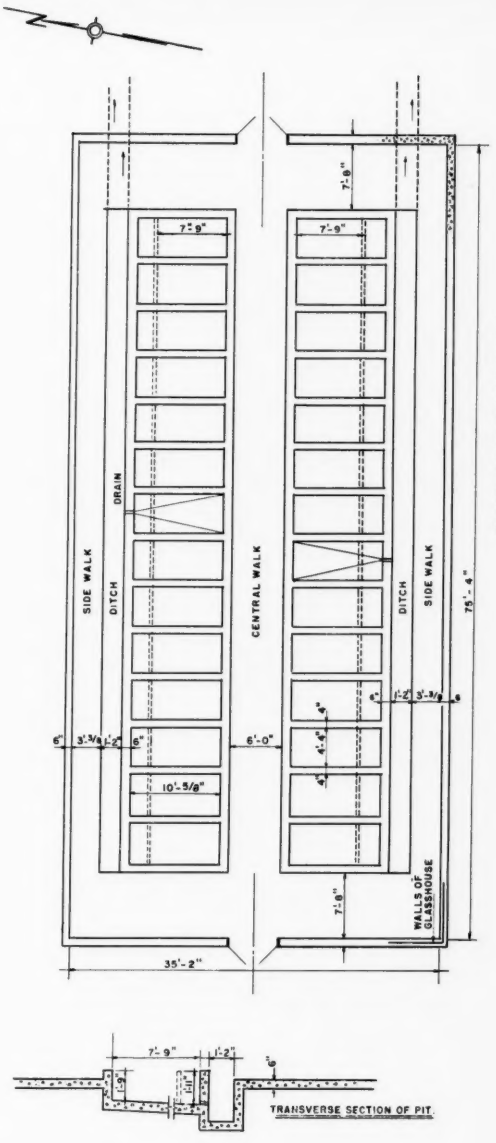


FIG. 1.—Layout of floor space in and around the 28 concrete pits in the glasshouse.

concrete cisterns, each with a capacity of 20,946 gallons, was used for pump irrigation of the crops.

Preliminary pot tests in sand were run with Hegari sorghum, buckwheat, beans, and sugarcane variety P.R. 900, to determine the most favorable nutrient concentration for these crops. Nineteen experiments with crops including sugarcane, beans, corn, sweetpotatoes, and cotton, were per-

FIG. 2.—View inside the glasshouse when corn plants were growing.

formed in the pits during the 15-year period between August 6, 1940, and July 12, 1955.

Germination and crop growth were abnormal under the glasshouse in the first six experiments, due mainly to uneven sunlight distribution and air-ventilation, and to high temperatures. Around 2:00 p.m. the temperatures varied 28 to 43° C. and around 6:00 a.m. from 15 to 17° C. The coefficients of variability of the crop data were too high, varying from 28 to 40 percent for sugarcane, beans, corn, and sweetpotatoes. To correct for temperature variations the screens and glasses were removed from the greenhouse in July 1946. The seventh and eighth crops then grew normally without the roof and side covers, but the former crop was affected by excess of rainfall and drainage. The length of each of the 28 pits was then reduced to 7 feet

9 inches, and its area to 0.00077 acre, by constructing a 2-inch concrete wall (fig. 1) at the drainage outlet (fig. 3). The glasses were replaced in the roof of the greenhouse and the sides were kept open after the eighth crop was harvested in February 1948. Normal growth and yields were obtained in the ninth corn crop and the coefficient of variability of the crop data was reduced to 21.8 percent. It was further reduced to 11.5 percent in the fifteenth crop, which was sugarcane.

FIG. 3.—Drainage outlets for the pits after construction of the 2-inch concrete wall.

Correlation studies between leaf composition of major nutrients and crop yields were initiated in May 1949 with the tenth crop. By that time the authors (*3*)[3] had developed rapid and precise photometric methods for foliar N-P-K analyses, and Capó (*5*) had developed the arc-tangent formula that expresses the quantitative relationship between yields and composition of the plant as follows:

$$Yr = A + B \text{ arc-tan percent } Nu$$

where $Yr$ is the relative yield, $A$ and $B$ are constants, and arc-tan percent $Nu$ is the arc whose tangent is the percentage of the respective nutrient of

[3] Italic numbers in parentheses refer to Literature Cited, p. 184.

the plant on a dry-matter basis. It was also known that nitrogen was the most important element that limited crop growth in Puerto Rico; that the soils (*1*) were well supplied with available phosphorus and potassium; that responses to phosphorus (*1*) were generally limited to short-time crops; and that few responses were obtained with potassium (*2*) used on long-time crops like sugarcane.

The 6-year (1949–55) data obtained in the last 10 experiments with the crops grown were then used for the correlation studies reported here.

## EXPERIMENTAL PROCEDURE

The strongly acid (pH 4.6), medium-to-fine (table 1), almost pure quartz sand (99 percent $SiO_2$) classified as St. Lucie fine sand (*7*) was used as the growth medium in the pits. This is the same sand used as raw material for glass-manufacturing in Puerto Rico. The sand used for the last two

TABLE 1.—*Mechanical composition of the quartz sand used in the experiments*

| Classification | Diameter limits | Percentage |
|---|---|---|
| | *Mm.* | |
| Fine gravel | 2.00–1.00 | 0.08 |
| Coarse sand | 1.00– .50 | 5.00 |
| Medium sand | .50– .25 | 46.64 |
| Fine sand | .25– .10 | 42.65 |
| Very fine sand | .10– .05 | 4.79 |
| Silt and clay | Less than– .05 | .84 |

experiments with cotton was alkaline (pH 8.0) owing to limestone and pieces of broken glass added in the glass factory from which the sand was bought.

A 3-inch layer of 800 pounds of thoroughly washed, noncalcareous, ½-inch, river gravel was placed on the bottom of each of the 28 pits, over which 3,000 pounds of the sand were spread.

The major elements studied were nitrogen, phosphorus, and potassium, respectively. Seven randomized treatments replicated four times were used. They consisted of variable unit concentrations of the element under study and maximum rates of other two elements. The unit concentration used per pit was 0.03 pound of N, 0.015 pound of $P_2O_5$, and 0.015 pound of $K_2O$.

Pure salts of the major nutrients required for each experiment were then added at the levels required for the different crops. The nitrogen was applied to the sand in the ratio of 90 parts of nitrate and 10 parts of ammonia, as proposed by Trealease and Trealease (*8*) for a medium around

pH 6.0. The ammonia was added as ammonium sulfate and the nitrate as tetrahydrated calcium nitrate. The phosphorus was applied as monobasic monohydrated calcium phosphate and the potassium as potassium sulfate. The calcium concentration was balanced with gypsum and calcium carbonate. The sulfur concentration was balanced with gypsum. Minor elements were also added in equal quantities to each pit. Examples of the major nutrient levels studied, the kinds and quantities of all salts added, and the concentrations of calcium, sulfur, and minor elements used for the

TABLE 2.—*Examples of major nutrient levels and quantities of principal salts added, in grams per pit*[1], *in the different treatments*

| Treatment No. | Nutrient levels[2] in units per pit | | | $(NH_4)_2SO_4$ | $Ca(NO_3)_2 \cdot 4H_2O$ | $Ca(H_2PO_4)_2 \cdot H_2O$ | $K_2SO_4$ | $CaCO_3$ | $CaSO_4 \cdot 2H_2O$ |
|---|---|---|---|---|---|---|---|---|---|
| | N | $P_2O_5$ | $K_2O$ | | | | | | |
| 1 | 3 | 15 | 22.2 | 19.25 | 309.68 | 181.23 | 279.45 | 336.66 | 73.28 |
| 2 | 6 | 15 | 22.2 | 38.50 | 619.36 | 181.23 | 279.45 | 220.30 | 47.63 |
| 3 | 9 | 5 | 22.2 | 57.75 | 929.04 | 60.41 | 279.45 | 151.30 | 23.09 |
| 4 | 9 | 10 | 22.2 | 57.75 | 929.04 | 120 82 | 279.45 | 127.32 | 23.09 |
| 5 | 9 | 15 | 7.4 | 57.75 | 929.04 | 181 '3 | 93.15 | 28.50 | 151.80 |
| 6 | 9 | 15 | 14.8 | 57.75 | 929.04 | 181.2[illegible] | 186.30 | 84.94 | 54.72 |
| 7 | 9 | 15 | 22.2 | 57.75 | 929.04 | 181.23 | 279.45 | 103.32 | 23.09 |

[1] Area of pit = 0.00077 A. Sand per pit = 3,000 lb.

[2] Nutrient levels are 0.03, 0.015, and 0.015 lb. per unit of N, $P_2O_5$, and $K_2O$, respectively. The N was added as $(NH_4)_2SO_4$ and $Ca(NO_3)_2 \cdot 4H_2O$ in the ratio of 90 parts $NO_3$ to 10 parts $NH_3$. All salts added were c.p. Grams per pit for other nutrients were: Ca—233.5, S—124.6 (from main and minor salts), Mg—39.9 as $MgSO_4 \cdot 7H_2O$, Fe—18.2 as $FeSO_4 \cdot 7H_2O$, Zn—1.1 as $ZnSO_4 \cdot 7H_2O$, Cu—0.4 as $CuSO_4 \cdot 5H_2O$, Mn—1.5 as $MnSO_4 \cdot 2H_2O$, B—0.2 as $H_3BO_3$.

sweet corn planted in the eighth experiment are reported in table 2. A similar procedure was followed with the other crops.

The total nitrate salt required for all the treatments in each experiment was dissolved in about 1 l. of distilled water and diluted to 2 l. in a volumetric flask. Aliquots representing the nitrates required per treatment and per pit were measured respectively, and poured into one sprinkler containing about 8 l. of rainwater. The solutions were then sprinkled over the sand in each corresponding pit and the sand was sprinkled again with water.

The quantity of each of the other nutrient salts required per treatment per pit was weighed separately, poured into an 8-l. enameled pail, and dissolved in rainwater. The solution of all the mixed salts was poured uniformly over the sand of each corresponding treatment, the salt residue was again dissolved in water, and the crop was irrigated with two more pails

filled with water. The pits filled with sand were now ready for planting the crop to be studied.

Corn grains were placed in a porous linen bag attached to a faucet and were soaked overnight under running water. Five corn seed were planted in each of 18 holes, adequately spaced in each pit, but only two plants were left per hole. Sugarcane seed were disinfected before planting by immersion in hot water at 52° C., for 20 minutes and then were cut crosswise in one-eye node sections. These sections were planted at the rate of one for each of four holes adequately spaced in each pit; extra ones germinated in a separate sand bed supplied material for replanting. The cotton seed were treated with a 1:1 solution of sulfuric acid for 30 minutes and then washed with tapwater. Seven cotton seed were planted in six shallow holes about ½-inch deep, adequately spaced in each pit. The germinated plants of corn or of cotton were thinned to two for each hole. Adequate attention was given to pest control. The crops were irrigated when needed after the drainage collected in each pit was poured back over the whole sand surface. Algae growth on the top sand layer was controlled by shallow cultivation with a wooden rake.

Leaf samples of the crops in each pit were taken for analysis at various growth intervals. Composite samples of the outer third portion of the fourth leaves were taken from the corn plants; of the third, fourth, fifth, and sixth leaves from the sugarcane plants; and of the third pair of young leaves from the first crop of cotton plants. The leaf samples were washed with water and prepared and analyzed for total nitrogen, phosphorus, and potassium by the method reported elsewhere (*3*, *5*). Data for yield of the crops were recorded at harvesttime.

The correlations between the arc-tangent (*5*) of the percentage of nitrogen, phosphorus, or potassium in the leaf at various growth intervals of 6 of the 10 crops studied and their relative yields were studied.

## RESULTS AND DISCUSSION

The dates of planting and harvesting for the corn, sugarcane, and cotton, the varieties used, and the crop age at harvesttime for the 10 sand-culture experiments performed from 1949 to 1955 are reported in table 3. The nutrient levels in units per pit used in those experiments are reported in table 4.

The mean N-P-K contents of the sweet corn leaves at various day-intervals are reported in tables 5, 6, and 7, respectively. The mean N and K contents of the dent corn leaves are reported in tables 8 and 9. The mean P and K contents of the sugarcane leaves at various month-intervals are reported in table 10 and those for N in table 11. The mean N contents of the cotton leaves are reported in table 12.

TABLE 3.—*Experiments, varieties, planting and harvesting dates, and age of crops studied in sand cultures from 1949 to 1955*

| Experiment No. | Crop and variety | Planting date | Harvesting date | Crop age in months |
|---|---|---|---|---|
| 10 | Sweet corn, USDA 34 | Feb. 9, 1949 | May 11, 1949 | 3.1 |
| 11 | do. | Aug. 6, 1949 | Oct. 19, 1949 | 2.4 |
| 12 | do. | Jan. 20, 1950 | Apr. 21, 1950 | 3.0 |
| 13 | do. | July 11, 1950 | Sept. 21, 1950 | 2.3 |
| 14 | do. | Nov. 10, 1950 | Jan. 31, 1951 | 2.7 |
| 15 | Sugarcane, M. 336 | June 7, 1951 | Feb. 11, 1952 | 8.1 |
| 16 | Dent corn, Mayorbela | Apr. 4, 1952 | June 27, 1952 | 2.8 |
| 17 | Sugarcane, M. 336 | Sept. 25, 1952 | Dec. 25, 1953 | 15.0 |
| 18, 19[1] | Cotton, Sea Island[1] | Aug. 27, 1954 | Feb. 25, July 12, 1955 | 6.0, 3.6 |

[1] 1 planting and 2 crops.

TABLE 4.—*Crops and levels of major nutrients studied in sand cultures from 1949 to 1955*

| Experiment No. | Crop | Nutrient levels in units[1] per pit of | | |
|---|---|---|---|---|
| | | Nitrogen, N | Phosphorus, $P_2O_5$ | Potassium, $K_2O$ |
| 10 | Sweet corn | 2, 4, 6 | 5, 10, 15 | 5, 10, 15 |
| 11 | do. | 1, 2, 3, 4, 5, 6, 7 | 15 | 15 |
| 12 | do. | 3.3 | 3, 6, 9, 12, 15, 18, 21 | 15 |
| 13 | do. | 3.3 | 9 | 1, 2, 3, 4, 5, 6, 7 |
| 14 | do. | 3.3 | 9 | 0, .5, 1, 2, 3, 4, 5 |
| 15 | Sugarcane | 6.2 | 1, 5, 9 | 1, 5, 9, 13 |
| 16 | Dent corn | 1.7, 2.6, 3.4, 4.3 | 9 | 0.5, 1.5, 2.5, 3.5 |
| 17 | Sugarcane | 1, 2.5, 4, 5.5, 7, 8.5, 10 | 9 | 13 |
| 18, 19 | Cotton | .4, .8, 1.2, 1.6, 2.0, 2.4, 2.8 | 4 | 10.3 |

[1] Nutrient levels are equivalent to 0.03 lb. N, 0.015 lb. $P_2O_5$, or 0.015 lb. $K_2O$, per unit, respectively. Nutrient levels in pounds per pit are converted to pounds per acre at a plow depth of 6⅔ inches (assumed to be equal to 2 million pounds of soil) by dividing by 0.00077 acre, the area of each pit. The quantity of sand in each pit corresponding to an acre-plow depth of 6⅔ inches equals 1,540 lb. of sand per pit.

TABLE 5.—*Mean nitrogen contents at different crop ages of USDA 34 sweet corn leaves grown at different nitrogen levels added to sand cultures*

| Experiment No. | Units[1] of N per pit | Percentage of N at ages indicated in days | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 65 |
| 11 | 1 | 2.99 | | 2.87 | | 2.10 | | 1.42 | 1.39 |
| 10 | 2 | | 3.94 | | 3.82 | | 2.56 | | |
| 11 | 2 | 3.37 | | 3.61 | | 2.76 | | 1.69 | 1.69 |
| 11 | 3 | 3.44 | | 3.63 | | 3.07 | | 2.00 | 2.03 |
| 10 | 4 | | 4.24 | | 4.25 | | 3.46 | | |
| 11 | 4 | 3.46 | | 3.80 | | 3.32 | | 2.34 | 2.29 |
| 11 | 5 | 3.68 | | 3.77 | | 3.38 | | 2.50 | 2.50 |
| 10 | 6 | | 4.23 | | 4.47 | | 3.72 | | |
| 11 | 6 | 3.74 | | 3.91 | | 3.41 | | 2.59 | 2.52 |
| 11 | 7 | 3.85 | | 4.12 | | 3.52 | | 2.61 | 2.62 |

[1] 1 unit equals 0.03 lb. N.

TABLE 6.—*Mean phosphorus contents at different crop ages in USDA 34 sweet corn leaves grown at different phosphorus levels added to sand cultures*

| Experiment No. | Units[1] $P_2O_5$ per pit | Percentage of P at ages indicated in days | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 18 | 25 | 30 | 37 | 40 | 45 | 50 | 59 | 65 |
| 12 | 3 | 0.13 | 0.19 | | 0.12 | | 0.17 | | 0.19 | 0.22 |
| 10 | 5 | | | 0.31 | | 0.18 | | 0.12 | | |
| 12 | 6 | .19 | .45 | | .33 | | .33 | | .30 | .31 |
| 12 | 9 | .24 | .64 | | .46 | | .42 | | .35 | .39 |
| 10 | 10 | | | .55 | | .48 | | .29 | | |
| 12 | 12 | .23 | .59 | | .46 | | .44 | | .40 | .41 |
| 10 | 15 | | | .62 | | .47 | | .36 | | |
| 12 | 15 | .26 | .67 | | .49 | | .47 | | .47 | .45 |
| 12 | 18 | .29 | .75 | | .56 | | .51 | | .46 | .46 |
| 12 | 21 | .24 | .70 | | .52 | | .52 | | .50 | .49 |

[1] 1 unit equals 0.015 lb. $P_2O_5$.

TABLE 7.—*Mean potassium contents at different crop ages of USDA 34 sweet corn leaves grown at different potassium levels added to sand cultures*

| Experiment No. | Units of $K_2O$ per pit | Percentage of K at ages indicated in days | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 18 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 65 | 66 |
| 14 | 0 | 1.34 | 2.66 | | 0.76 | | 1.46 | | 1.45 | 1.45 | |
| 14 | 0.5 | 2.50 | 4.28 | | 1.27 | | 1.47 | | 1.37 | 1.60 | |
| 13 | 1 | | | | 2.02 | | 2.60 | | 2.66 | | |
| 14 | 1 | 4.52 | 4.95 | | 1.73 | | 2.04 | | 2.08 | 2.18 | 2.99 |
| 13 | 2 | | | | 2.23 | | 3.25 | | 2.99 | | |
| 14 | 2 | 4.41 | 6.20 | | 2.16 | | 2.07 | | 2.90 | 2.25 | 3.12 |
| 13 | 3 | | | | 2.43 | | 3.73 | | 3.26 | | |
| 14 | 3 | 4.98 | 6.00 | | 2.67 | | 2.47 | | 2.74 | 2.48 | 3.62 |
| 13 | 4 | | | | 2.45 | | 3.53 | | 3.27 | | |
| 14 | 4 | 6.20 | 6.70 | | 1.94 | | 2.57 | | 3.07 | 2.38 | 4.17 |
| 10 | 5 | | | 4.44 | | 3.08 | | 2.84 | | | |
| 13 | 5 | | | | 2.48 | | 3.67 | | 2.67 | | 3.90 |
| 14 | 5 | 6.40 | 6.71 | | 2.60 | | 3.20 | | 3.15 | 2.67 | |
| 13 | 6 | | | | 2.65 | | 3.91 | | 3.17 | | 4.30 |
| 13 | 7 | | | | 2.69 | | 3.64 | | 3.15 | | 4.48 |
| 10 | 10 | | | 4.36 | | 3.58 | | 2.97 | | | |
| 10 | 15 | | | 4.96 | | 4.25 | | 3.13 | | | |

[1] 1 unit equals 0.015 lb. $K_2O$.

TABLE 8.—*Mean nitrogen contents at different crop ages of Mayorbela dent corn leaves grown at different nitrogen levels added to sand cultures in experiment 16*

| Units[1] of N per pit | Percentage of N at ages indicated in days | | |
|---|---|---|---|
| | 35 | 45 | 55 |
| 1.7 | 4.1 | 2.6 | 1.6 |
| 2.6 | 4.1 | 3.0 | 1.5 |
| 3.4 | 4.4 | 2.8 | 1.6 |
| 4.3 | 4.2 | 3.1 | 1.6 |

[1] 1 unit equals 0.03 lb. N.

TABLE 9.—*Mean potassium contents at different crop ages of Mayorbela dent corn leaves grown at different potassium levels added to sand cultures in experiment 16*

| Units[1] of $K_2O$ per pit | Percentage of K at ages indicated in days | | |
|---|---|---|---|
| | 35 | 45 | 55 |
| 0.5 | 1.4 | 1.2 | 1.4 |
| 1.5 | 1.7 | 1.6 | 1.3 |
| 2.5 | 2.1 | 1.6 | 1.6 |
| 3.5 | 2.3 | 1.6 | 1.6 |

[1] 1 unit equals 0.015 lb. $K_2O$.

TABLE 10.—*Mean phosphorus and potassium contents at different crop ages of M. 336 sugarcane leaves grown at different phosphorus and potassium levels added to sand cultures in experiment 15*

| Units[1] per pit of | | | Percentage of P at ages indicated in months | | | | | | Percentage of K at ages indicated in months | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | $P_2O_5$ | $K_2O$ | 2 | 3 | 4 | 5 | 6 | 7 | 2 | 3 | 4 | 5 | 7 |
| 6.2 | 1 | 13 | 0.13 | 0.08 | 0.15 | 0.12 | 0.10 | 0.07 | | | | | |
| 6.2 | 5 | 13 | .22 | .09 | .16 | .14 | .11 | .10 | | | | | |
| 6.2 | 9 | 1 | | | | | | | 1.83 | 1.59 | 1.22 | 2.69 | 1.50 |
| 6.2 | 9 | 5 | | | | | | | 2.81 | 2.37 | 2.37 | 2.56 | 1.81 |
| 6.2 | 9 | 9 | | | | | | | 2.60 | 2.78 | 1.98 | 2.21 | 1.77 |
| 6.2 | 9 | 13 | .43 | .19 | .20 | .16 | .12 | .09 | 2.55 | 2.94 | 2.04 | 2.34 | 1.76 |

[1] 1 unit equals 0.03 lb. N, and 0.015 lb. $P_2O_5$ or $K_2O$.

TABLE 11.—*Mean nitrogen contents at different crop ages of M. 336 sugarcane leaves grown at different nitrogen levels added to sand cultures in experiment 17*

| Units[1] of N per pit | Percentage of N at ages indicated in months | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| 1.0 | 2.93 | 1.33 | 1.28 | 1.16 | 1.09 | 1.27 | 1.43 | 1.43 | 1.40 | 1.12 | 1.30 | 1.01 | 1.18 |
| 2.5 | 3.07 | 1.95 | 1.74 | 1.52 | 1.16 | 1.22 | 1.51 | 1.49 | 1.37 | 1.14 | 1.39 | 1.19 | 1.15 |
| 4.0 | 2.96 | 2.03 | 1.88 | 1.54 | 1.27 | 1.39 | 1.45 | 1.31 | 1.39 | .99 | 1.11 | .95 | 1.07 |
| 5.5 | 2.92 | 1.91 | 1.90 | 1.82 | 1.53 | 1.43 | 1.71 | 1.51 | 1.38 | 1.11 | 1.25 | 1.08 | 1.03 |
| 7.0 | 3.03 | 1.99 | 1.98 | 1.69 | 1.70 | 1.60 | 1.78 | 1.61 | 1.39 | 1.20 | 1.32 | 1.19 | 1.31 |
| 8.5 | 3.00 | 2.12 | 2.05 | 1.92 | 1.69 | 1.75 | 1.79 | 1.64 | 1.53 | 1.25 | 1.39 | 1.29 | 1.20 |
| 10.0 | 3.10 | 2.14 | 2.00 | 1.91 | 1.82 | 1.80 | 1.92 | 1.68 | 1.65 | 1.35 | 1.47 | 1.28 | 1.29 |

[1] 1 unit equals 0.03 lb. N.

TABLE 12.—*Mean nitrogen contents at different crop ages of Sea Island cotton leaves grown at different nitrogen levels added to sand cultures in experiment 18*

| Units[1] per pit of | | | Percentage of N at ages indicated in months | | | | |
|---|---|---|---|---|---|---|---|
| N | $P_2O_5$ | $K_2O$ | 1 | 2 | 3 | 4 | 5 |
| 0.4 | 4 | 10.3 | 3.27 | 2.55 | 2.29 | 2.14 | 1.66 |
| .8 | 4 | 10.3 | 3.60 | 3.71 | 2.92 | 2.13 | 1.73 |
| 1.2 | 4 | 10.3 | 3.48 | 3.07 | 2.95 | 2.46 | 2.05 |
| 1.6 | 4 | 10.3 | 3.88 | 4.28 | 3.10 | 2.27 | 1.67 |
| 2.0 | 4 | 10.3 | 4.00 | 4.04 | 3.25 | 2.12 | 1.72 |
| 2.4 | 4 | 10.3 | 3.90 | 3.61 | 3.88 | 3.31 | 2.53 |
| 2.8 | 4 | 10.3 | 4.08 | 4.24 | 3.60 | 2.79 | 2.08 |

[1] 1 unit equals 0.03 lb. N, and 0.015 lb. $P_2O_5$ or $K_2O$.

TABLE 13.—*Mean yields of USDA-34 sweet corn grown at different N-P-K levels added to sand cultures, and least significant differences between treatment means*

| Experiment No. | Treatment No. | Units[1] per pit | | | Mean crop yield of 4 replicates in hundredweights of grain per acre |
|---|---|---|---|---|---|
| | | N | $P_2O_5$ | $K_2O$ | |
| 10 | 1 | 2 | 15 | 15 | 6.5 |
| | 2 | 4 | 15 | 15 | 17.9 |
| | 3 | 6 | 5 | 15 | 10.4 |
| | 4 | 6 | 10 | 15 | 31.0 |
| | 5 | 6 | 15 | 5 | 31.6 |
| | 6 | 6 | 15 | 10 | 28.8 |
| | 7 | 6 | 15 | 15 | 28.6 |
| L.S.D. between treatment means at: 1-percent point | | | | | 3.8 |
| 5-percent point | | | | | 2.7 |
| 11 | 1 | 1 | 15 | 15 | 1.7 |
| | 2 | 2 | 15 | 15 | 7.0 |
| | 3 | 3 | 15 | 15 | 4.4 |
| | 4 | 4 | 15 | 15 | 15.4 |
| | 5 | 5 | 15 | 15 | 15.3 |
| | 6 | 6 | 15 | 15 | 15.1 |
| | 7 | 7 | 15 | 15 | 12.6 |
| L.S.D. between treatment means at: 1-percent point | | | | | 2.7 |
| 5-percent point | | | | | 2.0 |
| 12 | 1 | 3.3 | 3 | 15 | 2.0 |
| | 2 | 3.3 | 6 | 15 | 18.4 |
| | 3 | 3.3 | 9 | 15 | 21.4 |
| | 4 | 3.3 | 12 | 15 | 20.9 |
| | 5 | 3.3 | 15 | 15 | 20.7 |
| | 6 | 3.3 | 18 | 15 | 20.6 |
| | 7 | 3.3 | 21 | 15 | 20.8 |
| L.S.D. between treatment means at: 1-percent point | | | | | 5.4 |
| 5-percent point | | | | | 3.8 |
| 13 | 1 | 3.3 | 9 | 1 | 9.8 |
| | 2 | 3.3 | 9 | 2 | 10.2 |
| | 3 | 3.3 | 9 | 3 | 9.5 |
| | 4 | 3.3 | 9 | 4 | 10.4 |
| | 5 | 3.3 | 9 | 5 | 10.4 |
| | 6 | 3.3 | 9 | 6 | 9.3 |
| | 7 | 3.3 | 9 | 7 | 12.4 |
| L.S.D. between treatment means at: 1-percent point | | | | | 3.28 |
| 5-percent point | | | | | 2.39 |

| Experiment No. | Treatment No. | Units[1] per pit | | | Mean crop yield of 4 replicates in hundredweights of grain per acre |
|---|---|---|---|---|---|
| | | N | $P_2O_5$ | $K_2O$ | |
| 14 | 1 | 3.3 | 9 | 0 | 1.8 |
| | 2 | 3.3 | 9 | 0.5 | 6.0 |
| | 3 | 3.3 | 9 | 1 | 7.3 |
| | 4 | 3.3 | 9 | 2 | 9.4 |
| | 5 | 3.3 | 9 | 3 | 8.1 |
| | 6 | 3.3 | 9 | 4 | 8.0 |
| | 7 | 3.3 | 9 | 5 | 8.2 |
| L.S.D. between treatment means at: 1-percent point | | | | | 2.5 |
| 5-percent point | | | | | 1.9 |

[1] 1 unit equals 0.03 lb. N, and 0.015 lb. $P_2O_5$ or $K_2O$.

TABLE 14.—*Mean yields of Mayorbela dent corn grown at different N-P-K levels added to sand cultures, and least significant differences between treatment means in experiment 16*

| Treatment No. | Units[1] per pit | | | Mean crop yield of 4 replicates in hundredweight of grain per acre |
|---|---|---|---|---|
| | N | $P_2O_5$ | $K_2O$ | |
| 1 | 1.7 | 9 | 3.5 | 11.2 |
| 2 | 2.6 | 9 | 3.5 | 15.7 |
| 3 | 3.4 | 9 | 3.5 | 18.6 |
| 4 | 4.3 | 9 | 0.5 | 8.8 |
| 5 | 4.3 | 9 | 1.5 | 14.3 |
| 6 | 4.3 | 9 | 2.5 | 18.7 |
| 7 | 4.3 | 9 | 3.5 | 21.1 |
| L.S.D. between treatment means: 1-percent point | | | | 4.6 |
| 5-percent point | | | | 3.4 |

[1] 1 unit equals 0.03 lb. N, and 0.015 lb. $P_2O_5$ or $K_2O$.

The mean yields of sweet corn, dent corn, sugarcane, and cotton are reported in tables 13, 14, 15, and 16. The sugarcane data include yields of cane, sugar, trash, and roots, and the percentage of sugar in cane. The cotton data include yields of lint and seed. The least significant differences between mean yields of the different nitrogen treatments are also reported in the appropriate tables.

There were highly significant yield responses of corn to nitrogen, phosphorus, and potassium (tables 13, 14). There were highly significant responses of sugarcane in the yields of cane, sugar, trash, and roots, when either N or P was added (table 15). There were highly significant yield

responses of cotton in yields of lint and seed when nitrogen was added (table 16).

Highly significant correlations (table 17) were obtained between relative yields of corn and respective arc-tangent N contents in their leaves for

TABLE 15.—*Mean cane, sugar, trash, and root yields, and percentage of sugar of M. 336 sugarcane grown at different N-P-K levels added to sand cultures, and least significant differences between treatment means*

| Experiment No. | Treatment No. | Units[1] per pit | | | Mean crop yield in tons per acre | | | | Percentage of sugar in cane |
|---|---|---|---|---|---|---|---|---|---|
| | | N | $P_2O_5$ | $K_2O$ | Cane | Sugar | Trash | Roots | |
| 15 | 1 | 6.2 | 1 | 13 | 3.5 | 0.29 | 5.4 | 1.3 | 7.8 |
| | 2 | 6.2 | 5 | 13 | 14.0 | 1.46 | 10.6 | 2.9 | 10.4 |
| | 3 | 6.2 | 9 | 1 | 13.3 | .77 | 10.7 | 2.2 | 5.5 |
| | 4 | 6.2 | 9 | 5 | 18.3 | 1.35 | 13.1 | 3.1 | 7.1 |
| | 5 | 6.2 | 9 | 9 | 18.2 | 1.46 | 11.7 | 3.0 | 8.0 |
| | 6 | 6.2 | 9 | 13 | 18.5 | 1.60 | 12.3 | 2.6 | 8.5 |
| L.S.D. between treatment means at: | | | | | | | | | |
| 1-percent point | | | | | 8.2 | 0.93 | 3.9 | 1.2 | 3.9 |
| 5-percent point | | | | | 5.9 | .67 | 2.8 | .9 | 2.8 |
| 17 | 1 | 1.0 | 9 | 13 | 20.0 | 2.1 | 5.8 | 4.0 | 10.3 |
| | 2 | 2.5 | 9 | 13 | 27.8 | 3.1 | 8.1 | 5.9 | 11.2 |
| | 3 | 4.0 | 9 | 13 | 50.1 | 5.9 | 11.9 | 8.4 | 11.9 |
| | 4 | 5.5 | 9 | 13 | 62.8 | 8.3 | 13.8 | 7.5 | 13.1 |
| | 5 | 7.0 | 9 | 13 | 73.8 | 10.0 | 16.1 | 8.1 | 13.7 |
| | 6 | 8.5 | 9 | 13 | 81.3 | 11.0 | 19.0 | 7.7 | 13.5 |
| | 7 | 10.0 | 9 | 13 | 83.1 | 10.7 | 19.0 | 7.3 | 12.9 |
| L.S.D. between treatment means at: | | | | | | | | | |
| 1-percent point | | | | | 13.2 | 1.4 | 4.0 | 3.3 | 1.9 |
| 5-percent point | | | | | 9.6 | 1.0 | 2.9 | 2.4 | 1.4 |

[1] 1 unit equals 0.03 lb. of N and 0.015 lb. of $P_2O_5$ or $K_2O$.

ages varying from 25 to 65 days, also for the arc-tangent P and K contents from 18 to 65 days.

Significant correlations (table 18) were also obtained between the relative yields of sugarcane and arc-tangent N contents at the age of 4 months, and highly significant correlations at the age of 5 months. The correlation at different ages between nitrogen and green- and dry-matter contents of leaves and yields of sugarcane in this experiment have already been published (*4*). Highly significant correlations were obtained also (table 18) for arc-tangent phosphorus contents when the sugarcane plants were 3 or

TABLE 16.—*Mean yields of Sea Island cotton grown at different nitrogen levels added to sand cultures, and least significant differences between treatment means*

| Experiment No. | Treatment No. | Units[1] per pit | | | Mean crop yields in hundredweights per acre | | |
|---|---|---|---|---|---|---|---|
| | | N | $P_2O_5$ | $K_2O$ | Lint Cotton | Cotton with seed | Seed |
| 18 | 1 | 0.4 | 4 | 10.3 | .62 | 1.74 | 1.12 |
| | 2 | .8 | 4 | 10.3 | 3.01 | 8.46 | 5.45 |
| | 3 | 1.2 | 4 | 10.3 | 1.88 | 5.46 | 3.57 |
| | 4 | 1.6 | 4 | 10.3 | 5.58 | 14.62 | 9.04 |
| | 5 | 2.0 | 4 | 10.3 | 4.75 | 12.46 | 7.69 |
| | 6 | 2.4 | 4 | 10.3 | 4.32 | 11.37 | 7.05 |
| | 7 | 2.8 | 4 | 10.3 | 6.57 | 17.35 | 10.74 |
| L.S.D. between treatment means at: 1-percent point | | | | | 4.69 | 12.22 | 7.54 |
| 5-percent point | | | | | 3.42 | 8.92 | 5.50 |
| 19 | 1 | 0.4 | 4 | 10.3 | 1.35 | 3.20 | 1.85 |
| | 2 | .8 | 4 | 10.3 | 1.22 | 2.99 | 1.77 |
| | 3 | 1.2 | 4 | 10.3 | 1.36 | 3.42 | 2.06 |
| | 4 | 1.6 | 4 | 10.3 | 1.51 | 3.71 | 2.20 |
| | 5 | 2.0 | 4 | 10.3 | 1.29 | 3.15 | 1.85 |
| | 6 | 2.4 | 4 | 10.3 | 2.71 | 6.76 | 4.05 |
| | 7 | 2.8 | 4 | 10.3 | 2.11 | 5.30 | 3.19 |
| L.S.D. between treatment means at: 1-percent point | | | | | 1.98 | 4.80 | 3.15 |
| 5-percent point | | | | | 1.45 | 3.51 | 2.30 |

[1] 1 unit equals 0.03 lb. N, and 0.015 lb. $P_2O_5$ or $K_2O$.

TABLE 17.—*Significance[1] of correlations between relative yields of sweet corn and the arc-tangent of N-P-K contents of its leaves for different crop ages*

| Experiment No. | Arc-tangent of— | Significance at crop ages indicated in days | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 18 | 25 | 30 | 35 | 37 | 40 | 45 | 50 | 55 | 59 | 65 |
| 10 | N | | | N.S. | | | ** | | ** | | | |
| | P | | | ** | | | ** | | * | | | |
| | K | | | N.S. | | | N.S. | | * | | | |
| 11 | N | | ** | | ** | | | ** | | ** | | ** |
| 12 | P | ** | ** | | | ** | | ** | | | * | ** |
| 14 | K | ** | ** | | ** | | | ** | | ** | | ** |

[1] N.S.—nonsignificant; **—significant at 1-percent point; *—significant at 5-percent point.

4 months old, but no significant correlations for arc-tangent potassium contents were obtained in this experiment.

TABLE 18.—*Significance*[1] *of correlations between relative yields of sugarcane and the arc-tangent of the N-P-K contents at different crop ages*

| Experiment No. | Correlation between relative yields and | Significance at crop ages indicated in months | | |
|---|---|---|---|---|
| | | 3 | 4 | 5 |
| 15 | Arc-tangent P | ** | ** | |
| 15 | Arc-tangent K | N.S. | N.S. | |
| 17 | Arc-tangent N | N.S. | * | ** |

[1] N.S.—nonsignificant; **—significant at 1-percent point; *—significant at 5-percent point.

## SUMMARY

The layout for 28 concrete pits is described herein where sand-culture and irrigation studies under cover have been carried in Puerto Rico since 1940 with certain crops: Sugarcane, beans, corn, sweetpotatoes, and cotton. The data for 10 experiments performed with sweet corn, dent corn, sugarcane, and cotton are reported. These data included N-P-K contents of the respective leaves at different crop ages and yields obtained by varying the N or P or K levels from deficient to sufficient values, but keeping other major and minor nutrients constant.

These experiments were important in showing that foliar contents of the major nutrients at specific crop ages can be used to predict or correlate with relative yield values. These correlations are not generally obtained with phosphorous and potassium in field experiments because the initial available phosphorus and potassium contents of the soil are usually high.

## RESUMEN

Este trabajo ilustra la manera usada en Puerto Rico, desde el 1940, de preparar recipientes de concreto para llevar a cabo estudios con diferentes cosechas, tales como caña de azúcar, habichuelas, maíz, batatas, y algodón bajo techo y con riego. Se informan, también, los resultados de 10 experimentos hechos en estos recipientes con maíz, caña de azúcar y algodón.

Los datos obtenidos de estos experimentos incluyen los contenidos de N-P-K de las hojas de las mencionadas cosechas a distintas edades y los rendimientos alcanzados con niveles variables de N, de P, o de K, empezando con una cantidad mínima deficiente hasta la máxima suficiente, pero en todos los casos añadiendo cantidades suficientes de todos los otros nutrientes.

Estos experimentos han sido de gran utilidad para demostrar que los análisis foliares en cuanto a N-P-K, a una edad específica, pueden predecir los rendimientos relativos o estar relacionados con los mismos. Estas correlaciones no se obtienen regularmente de los datos de los experimentos de campo, porque generalmente los contenidos de P y K en los suelos de Puerto Rico son altos.

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# Termite-Repellent Activity and Chemical Composition of West Indian Mahogany Wood, (*Swietenia Mahagoni* Jacq.) with Special Reference to the $P_2$ Fraction[1]

*Conrado F. Asenjo, Luis Amorós Marín, Wanda Torres, and Alice del Campillo*[2]

## INTRODUCTION AND OBJECTIVE

The studies of Wolcott (*1*)[3] on the resistance of different woods to the "polilla", as it is locally called, the West Indian dry-wood termite, *Cryptotermes brevis* Walker, confirmed what was already known through practical experience, *i.e.*, that the West Indian mahogany (*Swietenia mahagani* Jacq.) is one of the few commercially available woods very resistant to this insect.

In 1951, one of us (C.F.A.) isolated (*2*) from West Indian mahogany wood a crude waxy-white substance which rated very high as a termite repellent when assayed by the biological method of Wolcott. This initial encouraging result, as well as the fact that a perusal of the literature revealed no investigations on the chemistry of West Indian mahogany wood, prompted this study.

## MATERIAL AND METHODS

Three samples of West Indian mahogany were used. One sample was obtained from a local furniture shop, although it came originally from the Dominican Republic; it was used by Asenjo (*2*) and later by us. The other two samples were obtained in Haiti, through the courtesy of Ernst Wulff and Georges Deslandes of Port-au-Prince.

The first sample from the Dominican Republic was extracted, serially, with selective solvents, that is, first with petroleum ether (b.p. 30°–60° C.), and then, successively, with ethyl ether, acetone, 95-percent alcohol, water, 3-percent HCl, and 2-percent NaOH. The results of these extractions are

[1] A cooperative project with the Agricultural Experiment Station, University of Puerto Rico, Río Piedras, P. R.

[2] Professor, Assistant Professor, Assistant, and Instructor, respectively, Department of Biochemistry and Nutrition, School of Medicine, School of Tropical Medicine, University of Puerto Rico, San Juan 22, P. R.

[3] Italic numbers in parentheses refer to Literature Cited p. 195.

recorded in the following tabulation:

| *Solvent* | *Percentage extracted* |
|---|---|
| Petroleum ether (30°–60° C.) | 0.73 |
| Ethyl ether | .08 |
| Acetone | 2.80 |
| Alcohol, 95 percent | .30 |
| Water | 2.10 |
| HCl, 3 percent | .98 |
| NaOH, 2 percent, more than | 10.00 |

Each of the above extracts was assayed for termite-repellent activity by the Wolcott assay procedure (*3*)[4]. This test consisted briefly of submerging a standard Flamboyan (*Delonix regia*) block, of about 0.7 x 2 x 3 cm., for a period of 10 minutes in a solution of known concentration of the substance under test. After impregnation the blocks were dried in the sun and exposed in petri dishes to fresh live termites obtained from some infected piece of wood. Observations were made daily until the termites attacked the treated wood. The number of days after impregnation on which the block was attacked served as an index of repellency. By running this test using blocks impregnated with different concentrations of the substance assayed the minimum effective concentration could be determined.

The initial termite-repellent tests were carried out in the spring of 1951. The petroleum ether extract (PEE) was the only one of all those tested to exhibit promising termite-repellent activity.

### FRACTIONATION OF THE PETROLEUM ETHER EXTRACT

After the petroleum ether was removed by distillation, a viscous reddish-yellow oil remained, which deposited a waxy-white precipitate on standing at room temperature, but more at icebox temperature. This precipitate amounted to about 41 percent of the petroleum ether extractive and 0.3 percent of the original wood. The fractionation procedure used is indicated in the flow diagram on page 187.

Fractions $P_{2a}$, $P_3$, and $P_4$ had the same softening range and the same solubility as $P_2$. Also, all these fractions reacted with hot diluted aqueous NaOH, turning deep brown in color and developing an elusive aromatic odor. After saponification a waxlike residue with solubility properties similar to the original material (fraction $P_5$) was left. It was purified by reprecipitation from alcohol after being decolorized with charcoal (Darco). The softening range of this amorphous material was lower (90–96° C.) than that of the original fraction (see table 1).

[4] All termite-repellent assays reported in this paper were performed by George N. Wolcott, Entomologist, Agricultural Experiment Station, Río Piedras, P. R. Our sincere thanks to Dr. Wolcott who is now retired.

Mahogany wood
Extracted with petroleum ether (PE)
PE extract
Extracted wood
Viscous oil ($P_1$)
Waxy-white ppt. (WWP) m.p. (70–120° C.)
Refluxed twice with PE
PE-soluble part precipitated on standing in icebox ($P_3$) m.p. (70–120° C.)
PE-insoluble part ($P_2$) m.p. (70–120° C.)
Refluxed with more PE
PE-soluble part precipitated on standing in icebox ($P_4$) m.p. (70–120° C.)
PE-insoluble part m.p. (70–120° C.) $P_{2a}$
Refluxed with 0.1 N NaOH in water
Insoluble part ($P_5$) (m.p. 90–96° C.)
Soluble part acid in nature

Acidification of the alkaline liquor with HCl yielded a sticky, somewhat oily brown substance that floated in the aqueous medium and which was removed with petroleum ether in which it was soluble. Attempts to purify this acidic fraction and/or to prepare derivatives from it were unsuccessful.

## RESULTS

### TERMITE-REPELLENT ACTIVITY

In table 2 are tabulated the termite-repellent activites of the different fractions separated. Fractions $P_2$ and $P_3$, the first one in particular, ex-

TABLE 1.—*Termite-repellent activity by Wolcott assay procedure of fractions from the petroleum ether extract of West Indian Mahogany wood*

| Fraction | Days it took for termites to attack Flamboyan blocks that had been submerged for 10 minutes to dilutions of the fraction under test expressed in percentages of fraction | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.002 | 0.005 | 0.01 | 0.02 | 0.05 | 0.10 | 0.20 | 0.50 |
| $P_1$ | | | 8 | 9 | 10 | | | |
| $P_2$ | | 1,005 | 1,045 | 1,051 | 1,055 | | | |
| $P_3$ | | 314 | 320 | 325 | 327 | | | |
| $P_4$ | 30 | 33 | 37 | 39 | 41 | 47 | 66 | 135 |
| $P_5$ | 36 | 38 | 39 | 43 | 56 | | | |
| Second preparation of $P_2$ fraction obtained from the same original mahogany sample | 40 | 54 | 62 | 63 | 65 | 68 | | |
| Third preparation of $P_2$ fraction obtained from the Haitian mahogany | 17 | 28 | 52 | 54 | 66 | 75 | | |

hibited a high repellent activity, protecting Flamboyan blocks for over 1,000 days at a concentration of only 0.005 percent. However, other $P_2$ fractions prepared at later dates by the same procedure, exhibited much lower termite-repellent activity.

Another approach used to determine the effect of petroleum ether on the termite-repellent activity of mahogany was to exhaustively extract standard assay blocks of mahogany with petroleum ether for several days. When these blocks were exposed to live termites, they were attacked, thus indicating that extraction with petroleum ether in some way made mahogany wood susceptible to termite attack (fig. 1)[6].

[6] The original test was carried out by Dr. Wolcott. We repeated it and obtained similar results. Fig. 1 is a photograph of the test carried out by us.

### SOLUBILITY CURVE OF THE $P_2$ FRACTION

The solubility of the $P_2$ fraction in double-distilled petroleum ether (b.p. 30–60° C.) at 28° C. was of about 0.16 gm. per 100 ml. of solvent. Its solubility in normal heptane was of about the same order of magnitude as in petroleum ether.

In order to determine whether the $P_2$ fraction was composed of only one

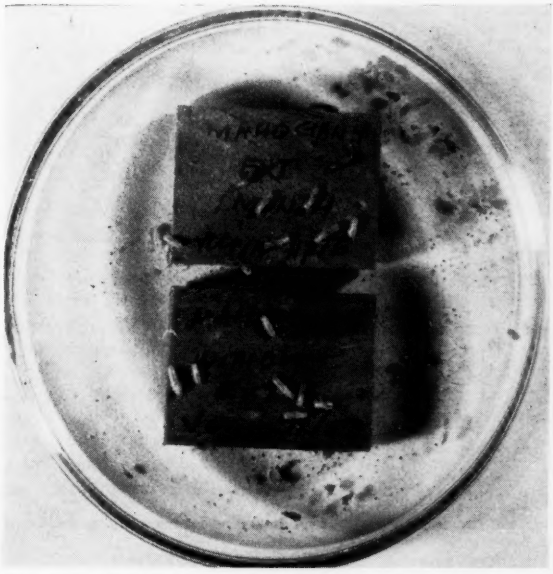

FIG. 1.—Mahogany blocks exposed to live termites. Upper block was extracted during 1 month with petroleum ether. Lower block was not extracted. The boring action of termites on the surface of the upper block is evident, especially the hole near the left-hand edge.

solid phase (a pure chemical compound) or of more than one solid phase (a mixture) the method of analysis of solubility curves described by Kunitz and Northrop (*4*) was used. This method consists briefly in determining the fraction of substance under investigation that will go into solution when increasing amounts of the solid substance are put in contact with an equal volume of the solvent used.

The above analysis applies only to mixtures which give curves having sharp breaks. When a curve with only one sharp break is obtained, it indicates that the substance under investigation is a pure homogeneous substance having, of course, only one solid phase. Additional sharp breaks

indicate more than one solid phase present in the substance. A smooth curve indicates a solid solution and cannot be analyzed by this method.

The solubility curve constructed using the $P_2$ fraction as solute and normal heptane as solvent (fig. 2) indicated that the $P_2$ fraction is probably a mixture of two major components and an additional minor one, thus indicating that the $P_2$ fraction is not a homogeneous substance.

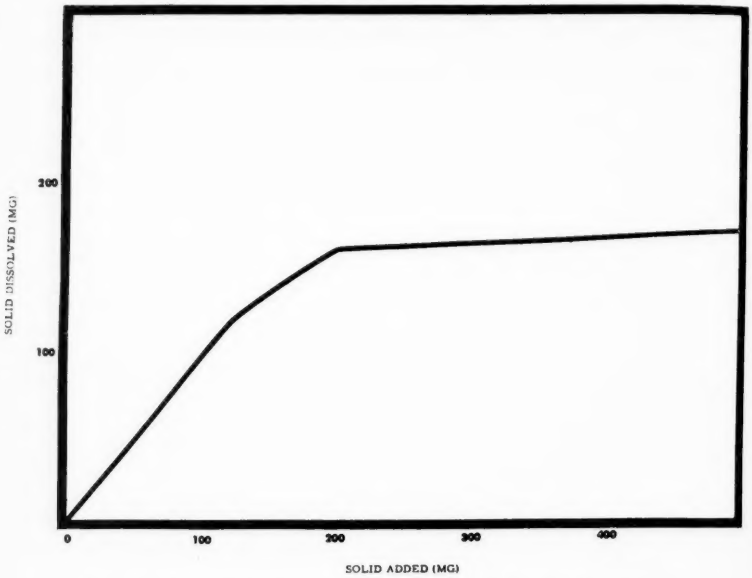


FIG. 2.—Solubility curve of the $P_2$ fraction in normal heptane.

## PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE $P_2$ FRACTION

Some of the principal physical and chemical characteristics of the $P_2$ fraction are reported in tables 2 and 3.

$P_2$ is highly soluble in all organic solvents except low molecular-weight hydrocarbons. It is slightly soluble with decomposition in dilute strong alkaline solutions and soluble without apparent decomposition in concentrated $H_2SO_4$ and $H_3PO_4$.

Tests made for halogens, nitrogen, and sulfur gave negative results. As the substance burns readily without leaving any ash, it was assumed to contain C, H, and O only. It has a negative rotation of about 21° and the iodine number indicates the presence of at least one unsaturated bond.

TABLE 2.—*Solubility of the $P_2$ fraction at 28° C.*

| Insoluble in— | Very slightly soluble in— | Slightly soluble in— | Soluble in— | Very soluble in— |
|---|---|---|---|---|
| $H_2O$ | Petroleum ether (30°–60° C.) | NaOH (dilute) | $H_2SO_4$ (concentrated) | Diethyl ether |
| HCl (dilute) | Normal-heptane | | $H_3PO_4$ (concentrated) | Acetone |
| $NaHCO_3$ (dilute) | | | | Dioxane |
| | | | | Cyclohexane |
| | | | | Chloroform |
| | | | | Benzene |
| | | | | Ethyl alcohol |
| | | | | Carbon disulfide |

TABLE 3.—*Some physical and chemical characteristics of the $P_2$ fraction from the extract of West Indian Mahogany wood*

| Physical and chemical characteristics | Values found | Remarks |
|---|---|---|
| Melting range (capillary tube) | 70–120° C. | Hot stage gave similar results |
| Average molecular weight: | | |
| a. Rast | 550 | Using camphor |
| b. Lowering of the freezing point | 500 | Using benzene as solvent |
| c. Raising of the boiling point | 800 | Using benzene as solvent |
| d. Calculated from iodine number assuming one double bond | 652 | |
| e. Calculated from saponification number assuming one carboxyl group | 540 | |
| $[\alpha]_D^{27°C.}$ in chloroform | −21.2 | |
| Elements present: | C, H, O only | Range of 13 analyses |
| Percent C | 60.67 | (59.25–61.56) |
| Percent H | 6.71 | (6.36–6.91) |
| Percent O (by difference) | 32.62 | (31.53–34.06) |
| Methoxyl content | 11 percent | |
| Iodine value | 38.9 | Hanus method |
| Saponification value | 105.0 | Using alcoholic KOH |
| Percentage of unsaponifiable | 12 | Using alcoholic KOH |
| Active hydrogen | 0.17 percent | |
| $(CH_3)$-C | 11.75 percent | |

## ALKALINE HYDROXAMIC REACTION

The $P_2$ fraction gave a positive hydroxamic reaction under alkaline conditions, a reaction typical of esters. On the other hand, the possibility of the presence of acid anhydrides was ruled out when the reaction mixture was incubated at a neutral pH (*5*) and no reaction took place. A modifica-

tion of chromatographic detection of acyl phosphates of Stadtman and Barker (*6*), based on the formation of hydroxamic acids and extraction and concentration of the same with alcohol and subsequent separation by paper chromatography, using water-saturated butanol as solvent, was used. A series of paper chromatograms consistently showed four definite spots as follows: One faint spot between $R_f$ 26–31; a second spot 51–53; a third 69-70; and a fourth faint spot at 85–88. As the first and fourth spots were visible before spraying with $FeCl_3$, they were probably due to some nonspecific reaction or impurity. Chromatograms were run using both Whatman No. 1 and No. 3 paper obtaining identical results. Spots No. 2 (Rf 51-53) and No. 3 ($R_f$ 69-70) seemed to be due to true acids, as they developed only after spraying with $FeCl_3$. In the literature (*6*) the $R_f$ for acetic and propionic acids are 52 and 65, respectively. Under our conditions known samples of acetic and propionic acids gave $R_f$ values of 49 and 70, respectively, therefore, seems that acetic and propionic acids are present in the form of esters in the $P_2$ fraction.

### ATTEMPTS TO PREPARE CRYSTALLINE ADDUCTS OF UREA AND THIOUREA

Urea forms crystalline complexes with almost any type of straight chain organic molecule, while thiourea acts in the same way in the presence of cyclic compounds (*7*).

No complexes were obtained when the $P_2$ fraction dissolved in methanol was mixed with urea or thiourea dissolved in the same solvent. Controls run at the same time with oleic acid and cyclohexane in methyl alcohol as solvent, yielded beautiful crystalline complexes with urea and thiourea, respectively.

### ULTRAVIOLET SPECTRUM

The ultraviolet absorption curve (Beckman DU spectrophotometer) of the $P_2$ fraction in ethanol (0.13 mg./ml.) showed a maximum at 217 and shoulders at 270 and 288. In cyclohexane the maximum was at 221 with shoulders also at 270 and 288.

The ultraviolet absorption curve of the $P_2$ fraction was not a very specific one and, therefore, did not aid greatly in characterizing the compounds present in this mixture.

### INFRARED SPECTRUM[6]

The infrared spectrum of the $P_2$ fraction was determined for us at the Eastern Utilization Branch of the Department of Agriculture in Phil-

[6] We are indebted to Dr. Thomas D. Fontaine, and Mr. J. S. Ard, Biologically Active Chemical Compounds Section, Agricultural Research Service, USDA, Philadelphia, Pa., for the infrared analysis.

adelphia. The most important facts suggested by the infrared spectrum of the $P_2$ fraction were the following:

1. Presumably an OH group is present which chelates intramolecularly. This is in accordance with the fact that the $P_2$ fraction is resistant to acetylation and that it has an active hydrogen as already indicated. However, it should be considered also that carbonyl over tones may cause a false interpretation of hydroxyls.
2. The compounds should be essentially aliphatic in nature.
3. There are indications of a carbonyl group, but one that is different from those in simple ketones or esters, and more like one in a tight environment like a pentacyclic ring having an unsaturated bond.
4. There are indications of one or more unsaturated bonds present. This is in agreement with the iodine number which indicates at least one double bond in one of the molecules.
5. There are indications of a possible Cis RCH=CHR group.
6. The presence of $CH_2$ groups like in a normal chain is also evident from the curve.
7. The presence of $(CH_3)$-CH is indicated. This was confirmed by actual analysis.
8. Possible presence of an ether group. This was confirmed by analysis.
9. A strong band at 1,227 $cm.^{-1}$ usually is dependable evidence of an ester group although the carbonyl band location at 1750 $cm.^{-1}$ is significantly different from that of noncyclic esters.
10. The general character of the infrared curve is negative for aromatic rings, anhydrides, and acetylenic groups. Also it suggests COOH and CHO radicals to be absent.

## DISCUSSION

The termite-repellent activity of mahogany wood seems to be present in a compound that is either extracted by petroleum ether from the wood or that, in some way, is inactivated *in situ* by this solvent. A solid waxy substance ($P_2$ fraction) isolated from the petroleum ether extractive, exhibited, at times, high termite-repellent activity, although large variations in repellent activity were observed from batch to batch.

From the available evidence, it seems that the different $P_2$ fractions studied contained variable quantities of a termite-repellent principle. The variable repellent activity exhibited by different $P_2$ preparations may be explained by the hypothesis that the termite-repellent principle is a very labile compound that suffers different degrees of chemical modifications during the isolation procedure.

A simple experiment that has offered substantial evidence to the effect that petroleum ether either removes the termite-repellent principle from

mahogany wood or, in some way, inactivates the same *in situ*, was the one in which mahogany blocks were subjected to exhaustive extraction with petroleum ether. The surface of these blocks became susceptible to termite attack.

The $P_2$ fraction, according to all the available evidence, is a mixture. The shape of the solubility curve in normal heptane suggested the presence of two major components, in addition to a minor one. The $P_2$ fraction contained only carbon, hydrogen, and oxygen, and its average percentage elemental composition from different samples of mahogany wood was very similar. The same is true in regard to the average molecular weight when determined by any one of the specific methods indicated.

As the $P_2$ fraction reacts with strong alkalies with decomposition and with the formation, at times, of a volatile substance of agreeable aromatic odor, the possibility of the presence of esters in this mixture has been highly suspected. This conclusion as to the chemical nature of the $P_2$ fraction receives further support from the interpretation of the infrared spectra and the hydroxamic acid reaction, both of which strongly point to the presence of esters in this fraction.

## SUMMARY

1. Serial extraction of West Indian mahogany wood with selective solvents was performed.

2. The petroleum ether extract was the only fraction to exhibit promising termite-repellent activity.

3. Mahogany blocks subjected to petroleum ether extraction became susceptible to termite attack.

4. A waxy substance ($P_2$ fraction) separated from the petroleum ether extract at times showed high termite-repellent activity. The termite-repellent principle in the $P_2$ fraction seems to be a labile one, as the termite-repellent activity varied greatly from batch to batch.

5. The $P_2$ fraction is a mixture of two major components and of at least a third minor one. As far as we have been able to determine this mixture, at the elemental level, contains carbon, hydrogen, and oxygen only, while esters have been the only compounds definitely characterized at the structural level.

## RESUMEN

1. Se llevó a cabo una extracción en serie de la madera de caoba con solventes selectivos.

2. El extracto de éter de petróleo demonstró la mayoractividad en cuanto a su acción repelente a la polilla.

3. Bloques de madera de caoba extraídos con éter de petróleo se tornaron susceptibles a los ataques de la polilla.

4. Una substancia cerosa (fracción $P_2$) separada del extracto de éter de petróleo demostró a veces una gran actividad repelente a la polilla. El principio repelente a la polilla de la fracción $P_2$ parece ser inestable, ya que su actividad repelente varió mucho de una prueba a otra.

5. La fracción $P_2$ parece ser una mezcla de dos componentes en mayor y untercero en menor cantidad. Hasta lo que hemos podido determinar esta mezcla, al nivel elemental, contiene solamente carbono, hidrógeno y oxígeno, mientras que al nivel estructural ésteres solamente han sido identificados.

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# Fruit and Vitamin C Production of Five- and Six-Year-Old Acerola Trees

*George C. Jackson and William Pennock*[1]

## INTRODUCTION AND OBJECTIVES

The acerola (*Malpighia punicifolia L.*) is now a commercial crop in Puerto Rico and promises to increase in importance in the near future. There are established today over 400 acres of commercial plantings and one processing plant has been in operation for a number of years. New plantings continue to be established at an accelerated rate.

Aside from observations made by Moscoso (*5*)[2], Aróstegui, *et al.* (*1*), Aróstegui and Pennock (*2*), and Ledin (*3, 4*), there is very limited information with respect to probable yields from commercial plantings here in Puerto Rico or elsewhere in the world.

Our purpose has been to obtain reliable data on expected commercial yields as well as on comparative clonal performance.

## MATERIALS AND METHODS

This study was carried out in a planting consisting of 163 trees which were set out in 1950. The planting comprised different selected clones in varying numbers, depending on their availability at the time of planting. The trees were set out at 16 x 16 foot spacing, and were fairly large plants derived from cuttings. They are now 6 to 10 feet tall with canopies 10 to 14 feet in diameter, depending on clonal growth habit. The trees may, therefore, be considered as having reached optimum or near-optimum size comensurate with the planting distance.

From now on some yearly pruning will necessarily have to be performed to facilitate mowing and other orchard-maintainance operations. To date pruning has been kept to a minimum and that done has been slight, mainly to improve tree shape during the first year after setting out. In subsequent years some small materials for propagation were cut from these trees. However, such branch-tip removal scarcely retarded their growth at all.

The recording of yields for each individual tree began in May 1955, at the start of the second cropping period for that year. It is unfortunate that the February crop for that year was past when this study was started. However, an estimate of this February crop has been made statistically. A

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[2] Italic numbers in parentheses refer to Literature Cited, pp. 205.

similar estimate was also made for the August crop in 1956. This latter crop was lost because of the hurricane of August 12.

Pickings were made at 2- and 3-day intervals. Whenever any dropped fruit was observed it was carefully picked up and included in the weighing. The fruit picked was either fully ripe or partially ripe, lacking an estimated 1, 2, or 3 days for complete ripeness. Doubtless commercial picking should be performed daily in order to get the fruit at its firm, almost ripe stage.

During the second year of this study samples consisting of 10 fruits were taken from the two highest yielding trees of each clone for vitamin C assay and for the determination of the refractive index of the juice. Samples were taken from each crop and were preserved by freezing for later determination. However, most of these samples were lost because of power failure following the hurricane of August 12. In consequence, only the samples of the October 1956 crop were actually properly assayed. For these assays the entire fruits were first osterized and then the determinations made from the filtered juice[3].

## EXPERIMENTAL RESULTS

Table 1 shows for 1955 and 1956, respectively, the 10 clones studied, the number of bearing trees of each clone in the planting, and the total yields per clone for each of the five crops during the year. Also shown are the annual clonal totals, the mean clonal yields per tree per year, and the mean clonal yield per tree per crop. A column of clonal totals from which the estimated crops have been excluded is also given.

In table 2 the yield data from both years are combined. To avoid tedious individual tree estimates, statistical analysis of clonal comparisons was performed using actual yields only[4]. In table 2, therefore, these actual yields are shown, as well as the yields which included estimates and which are therefore, more indicative of complete yearly performance.

In table 3 the clonal 2-year mean totals per tree are compared, and the statistically significant differences are shown. It will be seen therein that clone B15 outyielded all the other clones by a highly significant difference. In fact, it doubled or almost doubled the production of all the other clones except K7. It is unfortunate that there was only one tree of K7. Its yield data, therefore, lacked precision and were excluded from statistical comparison. Its indicated high yield in Puerto Rico must consequently be accepted with some reservations. This clone is the Florida Sweet which Ledin (*5*) has recently named and recommended for planting in Florida, where it is most vigorous and productive. It also grows vigorously in

[3] These assays were performed by the Food Technology Laboratory of this Station.
[4] All statistical analyses were performed by the Statistical Section of this Station.

TABLE 1.—*Data on fresh acerola fruits harvested at the Isabela Substation clonal planting during 1955 and 1956*

| Year and clones | Trees studied | Total fresh fruit harvested during different cropping periods | | | | | Clone totals with estimated crops excluded | Estimated crops included | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | February[1] | May | July | August[1] | October | | Total | Mean total per tree | Mean per tree per crop harvest |
| | *Number* | *Lb.* | *Lb.* | *Lb.* | *Lb.* | *Lb.* | *Lb.* | *Lb.* | *Lb.* | *Lb.* |
| 1955 | | | | | | | | | | |
| A1 | 57 | 301.4 | 1,201.4 | 1,160.1 | 1,451.2 | 1,048.8 | 4,861.5 | 5,162.9 | 90.6 | 18.1 |
| B15 | 21 | 65.7 | 774.4 | 938.6 | 987.2 | 1,096.6 | 3,796.8 | 3,862.5 | 183.9 | 36.8 |
| B17 | 16 | 40.7 | 275.9 | 421.1 | 407.9 | 342.2 | 1,447.1 | 1,487.8 | 93.0 | 18.6 |
| A2 | 16 | 104.4 | 346.8 | 252.9 | 364.1 | 163.2 | 1,127.0 | 1,231.4 | 77.0 | 15.4 |
| A10 | 15 | 68.9 | 215.5 | 244.9 | 327.0 | 328.4 | 1,115.8 | 1,184.7 | 79.0 | 15.8 |
| A4 | 11 | 23.4 | 322.7 | 137.9 | 395.2 | 144.3 | 1,000.1 | 1,023.5 | 93.0 | 18.6 |
| A21 | 13 | 32.9 | 179.7 | 191.0 | 293.1 | 280.9 | 944.7 | 977.6 | 75.2 | 15.0 |
| B9 | 7 | 9.1 | 116.3 | 146.4 | 174.0 | 142.4 | 579.1 | 588.2 | 84.0 | 16.8 |
| B2 | 6 | 20.0 | 202.3 | 90.0 | 196.0 | 50.1 | 538.4 | 558.4 | 93.1 | 18.6 |
| K7 | 1 | 11.3 | 33.6 | 44.7 | 37.1 | 27.1 | 142.5 | 153.8 | 153.8 | 30.8 |
| Total | 163 | 677.8 | 3,668.6 | 3,627.6 | 4,632.8 | 3,624.0 | 15,553.0 | 16,230.8 | 1,022.6 | 204.5 |
| Mean per tree | 1 | 4.2 | 22.5 | 22.3 | 28.4 | 22.2 | | 99.6 | 102.3 | 19.9 |
| 1956 | | | | | | | | | | |
| A1 | 57 | 412.0 | 2,127.8 | 1,971.5 | 1,984.0 | 1,206.2 | 5,717.5 | 7,701.5 | 135.1 | 27.0 |
| B15 | 21 | 89.8 | 1,298.9 | 1,129.8 | 1,349.6 | 1,267.7 | 3,786.2 | 5,135.8 | 244.6 | 48.9 |
| B17 | 16 | 55.7 | 408.5 | 207.6 | 557.6 | 271.2 | 943.0 | 1,500.6 | 93.8 | 18.8 |
| A2 | 16 | 142.8 | 767.3 | 543.9 | 497.8 | 542.4 | 1,996.4 | 2,494.2 | 155.9 | 31.2 |
| A10 | 15 | 94.2 | 294.1 | 176.1 | 447.0 | 203.0 | 767.4 | 1,214.4 | 80.9 | 16.2 |
| A4 | 11 | 32.0 | 482.2 | 175.1 | 540.3 | 90.0 | 779.3 | 1,319.6 | 120.0 | 24.0 |
| A21 | 13 | 45.0 | 379.1 | 165.2 | 400.7 | 240.3 | 829.6 | 1,230.3 | 94.6 | 18.9 |
| B9 | 7 | 12.5 | 176.7 | 66.1 | 237.9 | 157.9 | 413.2 | 651.1 | 93.0 | 18.6 |
| B2 | 6 | 27.3 | 267.5 | 114.3 | 268.0 | 122.8 | 531.9 | 799.9 | 133.3 | 26.7 |
| K7 | 1 | 15.4 | 35.7 | 30.5 | 50.7 | 9.8 | 91.4 | 142.1 | 142.1 | 28.4 |
| Total | 163 | 926.7 | 6,237.8 | 4,580.1 | 6,333.6 | 4,111.3 | 15,855.9 | 22,189.5 | 1,293.3 | 258.7 |
| Mean per tree | 1 | 5.7 | 38.3 | 28.1 | 38.8 | 25.2 | | 136.1 | 129.3 | 27.2 |

[1] Estimated statistically for August 1956.

Puerto Rico and quite probably is a higher yielder than many of our selections. It will also be observed in table 3 that clone A2 was a significantly higher yielder than A21 and A10, and that clone A1 also gave a significantly higher yield than A21 or A10. Clone B17, which has heretofore been regarded as our best clone, was actually outyielded by four of the clones in the experiment, but only B15 outyielded it significantly.

Table 4 gives the vitamin C determinations expressed as grams per 100 gm. of fresh fruit. These data are not significant at the 5-percent level. Table 5 is a summary of analyses of variance. As may be seen, the F

TABLE 2.—*Combined acerola yield data from clonal planting at the Isabela Substation for 1955 and 1956*

| Clones | Trees studied | Data excluding estimates | | | | Data including estimates | | |
|---|---|---|---|---|---|---|---|---|
| | | Totals from table 1 | Harvested August 9, 1956[1] | 2-year total | Mean 2-year total per tree | 2-year total | Mean 2-year total per tree | Mean per year per tree |
| | *Number* | *Lb.* | *Lb.* | *Lb.* | *Lb.* | *Lb.* | *Lb.* | *Lb.* |
| B15 | 21 | 7,583.0 | 48.8 | 7,631.8 | 363.4 | 8,998.3 | 428.5 | 214.3 |
| A2 | 16 | 3,123.4 | 23.1 | 3,146.5 | 196.7 | 3,725.6 | 232.9 | 116.5 |
| A1 | 57 | 10,579.0 | 215.6 | 10,794.6 | 189.4 | 12,864.4 | 225.7 | 112.9 |
| B2 | 6 | 1,070.3 | 2.7 | 1,073.0 | 178.8 | 1,358.3 | 226.4 | 113.2 |
| A4 | 11 | 1,779.4 | 0 | 1,779.2 | 161.7 | 2,343.1 | 213.0 | 106.5 |
| B17 | 16 | 2,390.1 | 19.5 | 2,409.6 | 150.6 | 2,988.4 | 186.8 | 93.4 |
| B9 | 7 | 992.3 | 5.3 | 997.6 | 142.5 | 1,239.3 | 177.0 | 88.5 |
| A21 | 13 | 1,774.3 | 7.7 | 1,782.0 | 137.1 | 2,207.9 | 169.8 | 84.9 |
| A10 | 15 | 1,883.2 | 4.5 | 1,887.7 | 125.8 | 2,399.1 | 159.9 | 80.0 |
| K7 | 1 | 233.9 | 0 | 233.9 | 233.9 | 295.9 | 295.9 | 148.0 |

[1] Only 1 harvest was made in August 1956, which was followed by the hurricane of Aug. 12, 1956. These data excluded from table 1 are shown here to explain mean clonal totals later used for statistical comparisons.

value of 2.61 is just short of the required value for 5-percent level of significance which is 2.85. Though just short of the usually accepted standard of significance, the data are not entirely untrustworthy. Since the data are the only available index of clonal vitamin C content, we have used them in estimating the annual production of vitamin C per tree as is shown in table 6. As may be seen therein, clone B15 exceeded all the other clones in vitamin C production. Clone B17, on the other hand, appears to produce less vitamin C than most of the other clones except A10.

## DISCUSSION

As may be seen in table 1, five well-defined bearing periods occurred during 1955 and 1956. Since the acerola has only recently become a commer-

TABLE 3.—*A 2-way comparison showing the differences between clonal mean totals, as well as the differences required for 1-percent and 5-percent levels of significance, using pounds of fresh acerola fruit yielded by the clonal planting at the Isabela Substation*[1]

| Clones | 2-year mean[2] totals | Item | B15 | A2 | A1 | B2 | A4 | B17 | B9 | A21 |
|---|---|---|---|---|---|---|---|---|---|---|
| 2-year totals per tree | | | 363.42 | 196.66 | 189.38 | 178.83 | 161.76 | 150.61 | 142.51 | 137.08 |
| A2 | 196.66 | Actual difference | 166.76** | | | | | | | |
| | | Required for 1-percent level | 76.24 | | | | | | | |
| | | Required for 5-percent level | | | | | | | | |
| A1 | 189.38 | Actual difference | 174.04** | 7.28 | | | | | | |
| | | Required for 1-percent level | 60.03 | | | | | | | |
| | | Required for 5-percent level | | 40.25 | | | | | | |
| B2 | 178.83 | Actual difference | 184.59** | 17.83 | 10.55 | | | | | |
| | | Required for 1-percent level | 94.52 | | | | | | | |
| | | Required for 5-percent level | | 61.61 | 51.72 | | | | | |
| A4 | 161.76 | Actual difference | 201.66** | 34.90 | 27.62 | 17.07 | | | | |
| | | Required for 1-percent level | 95.49 | | | | | | | |
| | | Required for 5-percent level | | 65.75 | 56.44 | 73.29 | | | | |
| B17 | 150.61 | Actual difference | 212.81** | 46.05 | 38.77 | 28.22 | 11.15 | | | |
| | | Required for 1-percent level | 115.78 | | | | | | | |
| | | Required for 5-percent level | | 82.03 | 74.78 | 88.00 | 91.09 | | | |
| B9 | 142.51 | Actual difference | 220.91** | 54.15 | 46.87 | 36.32 | 19.25 | 8.10 | | |
| | | Required for 1-percent level | 157.16 | | | | | | | |
| | | Required for 5-percent level | | 104.06 | 98.53 | 109.20 | 111.36 | 121.48 | | |
| A21 | 137.08 | Actual difference | 226.34** | 59.58* | 52.30* | 41.75 | 24.68 | 13.53 | 5.43 | |
| | | Required for 1-percent level | 72.90 | 77.55 | 61.62 | | | | | |
| | | Required for 5-percent level | | 55.65 | 44.26 | 64.33 | 68.29 | 84.06 | 105.70 | |
| A10 | 125.85 | Actual difference | 237.57** | 70.81* | 63.53** | 52.98 | 35.91 | 24.76 | 16.66 | 11.23 |
| | | Required for 1-percent level | 75.70 | 72.04 | 54.54 | | | | | |
| | | Required for 5-percent level | | 52.00 | 39.56 | 61.20 | 65.35 | 81.71 | 103.84 | 55.17 |

[1] * Denotes significance at the 5-percent level; ** denotes significance at the 1-percent level in all cases.
[2] The per tree mean totals here compared are those which do not include estimates shown in table 2.

TABLE 4.—*The vitamin C content of acerola-fruit samples taken October 1956 from some of the highest yielding trees of each clone at Isabela Substation clonal planting, expressed as grams per 100 gm. of fresh fruit*

| Tree sampled | Vitamin C content (grams) per 100 gm. fresh fruit for the different clones | | | | | | | | | | Totals |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | B17 | B15 | A2 | B2 | A1 | A4 | B9 | A21 | A10 | K7 | |
| 1st | 1.97 | 2.12 | 2.71 | 2.13 | 2.22 | 1.63 | 2.18 | 2.25 | 2.50 | 1.79 | 21.50 |
| 2nd | 2.03 | 2.36 | 2.49 | 1.65 | 1.95 | 1.52 | 2.68 | 2.75 | 1.81 | — | 19.24 |
| 3d | 1.93 | 2.36 | — | — | — | — | — | — | — | — | 4.29 |
| 4th | 1.90 | — | — | — | — | — | — | — | — | — | 1.90 |
| Total | 7.83 | 6.84 | 5.20 | 3.78 | 4.17 | 3.15 | 4.86 | 5.00 | 4.31 | 1.79 | 46.93 |
| Means | 1.96 | 2.28 | 2.60 | 1.89 | 2.09 | 1.58 | 2.43 | 2.50 | 2.16 | 1.79 | 2.13 |

TABLE 5.—*Summary of analyses of variance performed on data pertaining to vitamin C content of fruit from acerola trees in the Isabela Substation clonal planting*

| Source | D.F. | Sums of squares | Variance | F value |
|---|---|---|---|---|
| Total | 21 | 2.6519 | | |
| Between clones | 9 | 1.7542 | 0.1949 | 2.61 |
| Within clones | 12 | .8977 | .0748 | |
| Value at the 5-percent level of significance | | | | 2.85 |

TABLE 6.—*The mean annual yield of acerola fruit per tree, the vitamin C content of the fresh fruit, and the estimated total yield of vitamin C per tree for clonal planting at Isabela Substation*

| Clone | Trees | Annual fruit yield per tree | Content of vitamin C based on fruit weight | Annual production of vitamin C per tree |
|---|---|---|---|---|
| | *Number* | *Lb.* | *Per cent* | *Lb.* |
| B15 | 21 | 214 | 2.28 | 4.88 |
| A2 | 16 | 116 | 2.60 | 3.02 |
| K7 | 1 | 148 | 1.79 | 2.65 |
| A1 | 57 | 113 | 2.09 | 2.36 |
| B9 | 7 | 89 | 2.43 | 2.16 |
| B2 | 6 | 113 | 1.89 | 2.14 |
| A21 | 13 | 85 | 2.50 | 2.13 |
| B17 | 16 | 93 | 1.96 | 1.82 |
| A10 | 15 | 80 | 2.16 | 1.73 |
| A4 | 11 | 107 | 1.58 | 1.69 |
| Weighted means | | 118 | 2.17 | 2.56 |

1 * Denotes significance at the 5-percent level; ** denotes significance at the 1-percent level in all cases.
2 The per tree mean totals here compared are those which do not include estimates shown in table 2.

cial crop and its bearing habits have not been carefully observed and reported, we have considered it desirable to examine the data with the view of generalizing regarding the periodicity of fruiting throughout the year.

Table 7 shows the bearing and nonbearing periods for 1955 and 1956, giving the dates as well as the number of days included in each period. As can be seen in table 7 the data pertaining to duration of the resting peri-

TABLE 7.—*The bearing and nonbearing periods (days) of acerolas at the Isabela clonal planting during 1955 and 1956*[1]

| 1955 | | | 1956 | | |
|---|---|---|---|---|---|
| Resting periods | Bearing periods | Dates (inclusive) | Resting periods | Bearing periods | Dates (inclusive) |
| *Days* | *Days* | | *Days* | *Days* | |
| 113 | | Nov. 1–Feb. 21 | 110 | | Nov. 1–Feb. 18 |
| | 7 | Feb. 22–Feb. 28[2] | | 6 | Feb. 19–Feb. 24 |
| 79 | | Mar. 1–May 18 | 65 | | Feb. 25–Apr. 29 |
| | 20 | May 19–June 7 | | 17 | Apr. 30–May 16 |
| 27 | | June 8–July 4 | 33 | | May 17–June 18 |
| | 18 | July 5–July 22 | | 15 | June 19–July 3 |
| 32 | | July 23–Aug. 23 | 36 | | July 4–Aug. 8 |
| | 24 | Aug. 24–Sept. 16 | | 22 | Aug. 9–Aug. 30[3] |
| 16 | | Sept. 17–Oct. 2 | 26 | | Aug. 31–Sept. 25 |
| | 29 | Oct. 3–Oct. 31 | | 28 | Sept. 26–Oct. 23 |
| | | | 8[4] | | Oct. 24–Oct. 31 |
| 267[5] | 98[5] | | 278[5] | 88[5] | |

[1] The years are shown as starting on November 1, 1954 and 1955, presumably the first day of the resting period following the last harvest of the previous year.

[2] This period was not actually recorded. The length of the period in days was estimated statistically and the dates set down as could best be recalled from memory. It is substantially correct, plus or minus a few days.

[3] Aug. 9 was the date of the first and only picking made during this bearing period. The hurricane of Aug. 12 destroyed the remainder of the crop. The length of this cropping period was estimated statistically.

[4] Period to complete year on Oct. 31, 1956.

[5] Totals.

ods on the one hand, and to the duration of the bearing periods on the other, appear to follow a well-defined seasonal pattern which is similar for both years. The length of the resting period started at about 112 days, decreased rapidly at first to about 70 days and then tapered off by gradually decreasing differences to a length of about 21 days. These data suggest a parabolic decrease in the length of the resting period for the year starting on November 1. The bearing periods, on the other hand, started at about 7 days duration and increased roughly linearly to a duration of

about 28 days. However, since in both cases we are dealing with a recurring yearly phenomenon, an S-type curve would be required to fit the data applying to a period slightly longer than a year. The linear increase in the length of the bearing period should be regarded as the central section of a rather flat S curve.

Despite the suggested curves, however, the data are obviously too skimpy to provide the best fitting curves with acceptable standards of precision. Pending possible future corroboration, the information available at present on the pattern of production is as follows:

1. At the Isabela Substation in Puerto Rico, 10 selected clones of acerola produced in five well-defined cropping periods during the year 1955 and again in 1956. These cropping periods lasted from 1 to 4 weeks, during which successive fruit production was roughly continuous on a day-to-day basis for most trees. During the longest periods ripe fruit and bloom were frequently observed on the trees coincidentally.

2. In both years the four longest and most productive periods, accounting for 96 percent of the fruit, occurred during the months of May to October, inclusive. This coincides with relatively warm weather and abundant rainfall. If we allow a time lag of about 4 weeks for flower initiation, plus the known interval of 3 weeks from open flower to ripe fruit, it appears to coincide also with bloom induction which occurred after the spring equinox and before the autumm equinox. Fruit production during these months, therefore, corresponds to bloom induction during day-lengths of 12 to 13 hours.

3. A short bearing period lasting 1 week occurred in February of both years, when production amounted to only 4 percent of the yearly total. February is a relatively cold dry month in Puerto Rico and its corresponding bloom-induction period took place during the shortest days of the year having a few minutes more than 11 hours of daylight.

It would appear, therefore, that day length, within the Puerto Rican limits of variation, does not exert a clear-cut influence on bloom induction in acerolas. It may well be that a completely nonproductive winter may occur at a more northerly latitude. We believe such is the case in Dade County, Fla., where day-lengths in winter are reduced to as low as 10½ hours. However, this could be attributable to factors other than length of day.

4. Despite the recurrence of five general bearing periods during each of the two years of this study, there were some individual trees which only had four such periods, and a few were observed to have a few fruit in December 1956. These fruits were not harvested because so few, but they suggest the possibility that six bearing periods per year may occur in some instances. Such erratic behavior with respect to periodicity of bloom was observed to be much more frequent in an adjacent seedling planting which

was harvested during these same years, but for which no records were kept. It seems probable, therefore, that some clones may be found with a tendency to bloom more frequently during the year than others. Our present selections did not show much difference in this respect.

As regards probable yields from commercial production, the weighted mean obtained for all clones over the two years is doubtless our best estimate. As shown in table 6, it amounted to 118 pounds of fruit with a vitamin C content of 2.17 percent, or an estimated per tree per year yield of 2.56 pounds of vitamin C. However, it must be remembered that most of the clones included in this study were originally selected because of high content of vitamin C, and that their average is probably higher than the content of fruit in most commercial plantings. We believe, therefore, that 2 percent is a better estimate of vitamin C content of the fruit for commercial plantings in Puerto Rico.

On a per acre basis then, at 16 x 16-foot spacing, there would be 170 trees per acre, which would yield roughly 10 tons of fruit containing approximately 400 pounds of vitamin C. Because of the large size of these trees, and the need of holding them to present size by pruning, future yields of this planting will increase but slightly if at all. At wider spacing doubtless the trees could be allowed to attain a larger size, and would yield more individually, but there would be fewer trees per acre in rough compensation. These estimates, therefore, may be regarded as applying to an orchard in full production.

Regarding clonal superiority for commercial purposes: The data herein presented show beyond reasonable doubt that clone B15, under the conditions of this experiment, greatly outyielded all the others in terms of both fresh fruit and vitamin C. Since these conditions are presumably similar in their important essentials to those where acerolas are gown commercially on the Island, we strongly recommend clone B15 for commercial planting in Puerto Rico. Barring some possible undiscovered regional factor which might adversely affect its relative performance, the use of this clone would be expected to more than double the above estimated yields. The Florida Sweet (K7) is undoubtedly an outstanding clone for home planting and consumption as fresh fruit.

## SUMMARY

1. The production records obtained from 163 individual acerola trees belonging to 10 selected clones at the Isabela Substation are shown for the years 1955 and 1956. The trees were 5 and 6 years old from time of setting out as large, well-rooted cuttings.

2. The trees bore fruit during five well-defined cropping periods, which consisted of from 1 to 4 weeks of continuous production. These bearing

periods were interspersed with resting periods which varied from 2 to 16 weeks.

3. The four longest bearing periods, accounting for 96 percent of the yearly fruit total, occurred during the months of May to October, inclusive.

4. The yearly yield per acre of a commercial planting in Puerto Rico is estimated at 10 tons of fruit containing 435 pounds of vitamin C.

5. The most productive clone, now strongly recommended for commercial plantings, was B15 which more than doubled the above estimates. The Florida Sweet (K7), a selection made at Homestead, Fla., gave a high yield of fruit which had a relatively low vitamin content. It is rated as an outstanding clone for consumption as fresh fruit.

## RESUMEN

1. Se presentan en este trabajo datos sobre la producción anual de 163 árboles seleccionados de 10 variedades de acerola. Estos árboles fueron propagados asexualmente y sembrados en la Subestación de Isabela. Tenían 5 y 6 años de edad al ser cosechados en 1955 y 1956.

2. En ambos años la fruta se recogió durante 5 distintas épocas de cosecha cuya duración fué de una a cuatro semanas de producción continua. Entre cosechas hubo intervalos de descanso que duraron de 2 a 16 semanas.

3. Las cuatro cosechas mayores, cuyo total corresponde al 96 porciento de toda la fruta cosechada durante el año completo, ocurrieron durante los meses de mayo a octubre, inclusive.

4. El rendimiento anual para siembras comerciales en Puerto Rico se calcula en 10 toneladas de fruta por cuerda con un contenido de 400 libras de vitamina C.

5. La variedad clonal de mayor rendimiento fué la B15, la cual superó a todas las otras por un margen altamente significativo. De sembrarse esta variedad podrían doblarse los rendimientos arriba indicados. La variedad Florida Sweet (K7) dió un alto rendimiento de fruta fresca con un contenido relativamente bajo de vitamina C.

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(*Continued from inside front cover*)

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